PAULA DE AZEVEDO OLIVEIRA MILANEZ

# A FRUTOSE-1, 6-BIFOSFATO REDUZ DOR NEUROPÁTICA VIA ATIVAÇÃO DOS RECEPTORES DE ADENOSINA A1 E A2A: PARTICIPAÇÃO DA VIA DE SINALIZAÇÃO NO/GMPc/PKG/KATP

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Dissertação apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina, como requisito para obtenção do título de Mestre em Patologia Experimental.
Orientador: Prof. Dr. Waldiceu Aparecido Verri Junior

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Londrina, 12 de dezembro de 2011.

Ao meu marido, Rodrigo, meu eterno companheiro e o grande amor da minha vida.

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*"Toda a ciência provém da dor.*
*A dor procura sempre a causa das coisas,*
*enquanto o bem-estar se inclina a estar quieto*
*e a não olhar para trás."*

*Stefan Zweig*

MILANEZ, Paula de Azevedo Oliveira. **A Frutose-1, 6-Bifosfato reduz dor neuropática via ativação dos receptores de Adenosina A1 e A2A:** participação da via de sinalização NO/GMPc/PKG/KATP. 2011. 65f. Dissertação (Mestrado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina, 2011.

## RESUMO

Existem várias terapias para o controle da dor neuropática, mas os tratamentos atuais têm pouca eficiência e apresentam muitos efeitos colaterais, por isso novas alternativas terapêuticas são necessárias. A Frutose-1, 6-bifosfato (FBP) é um intermediário da via glicolítica que tem várias ações farmacológicas e, recentemente, foi demonstrado o seu efeito anti-hiperalgésico em modelo de dor inflamatória aguda via produção de adenosina. Curiosamente, a adenosina e agonistas do seus receptores A1 e A2A têm efeitos anti-hiperalgésicos em modelos de dor neuropática. Assim, investigamos o efeito anti-hiperalgésico e o mecanismo de ação da FBP em um modelo de dor neuropática induzido por constrição crônica (CCI) do nervo ciático de camundongos Swiss. Sete dias após a cirurgia, os animais foram tratados com diferentes doses de FBP ou adenosina por via oral ou intratecal. Além disso, os tratamentos da FBP e adenosina, por via oral, foram associados a administração de antagonistas dos receptores de adenosina A1 ou A2A, nas vias intraplantar ou intratecal. Por fim, o tratamento por via oral da FBP foi associado a inibidores da via de sinalização do óxido nítrico/GMPcíclico/Proteína quinase G/canais de potássio sensíveis ao ATP (NO/GMPc/PKG /KATP), que já foi demonstrada como via do mecanismo de ação da adenosina. A hiperalgesia mecânica foi avaliada por uma versão eletrônica dos filamentos de von Frey 1, 3, 5 e 7 horas após os tratamentos. A FBP e a adenosina inibiram a hiperalgesia mecânica induzida pela CCI de maneira similar. O mecanismo de ação da FBP parece ser dependente da produção de adenosina, já que seu efeito foi inibido pelos antagonistas dos receptores de adenosina A1 e A2A; além disso, o tratamento com a FBP promove anti-hiperalgesia através da via de sinalização NO/GMPc/PKG/KATP de forma semelhante à adenosina. Assim, demonstrou-se que a FBP tem benefício terapêutico na redução da dor neuropática e que seus mecanismos envolvem a ativação de receptores de adenosina A1 e A2A e a via de sinalização NO/GMPc/PKG/KATP.

**Palavras-chave:** Hiperalgesia. Dor neuropática. Frutose-1,6-bifosfato. Adenosina. Receptores de adenosina. Óxido nítrico.

MILANEZ, Paula de Azevedo Oliveira. **Fructose-1, 6-Bisphosphate reduces neuropathic pain activating Adenosine A1 and A2A receptors:** role of NO/cGMP/PKG/KATP signaling pathway. 2011. 65f. Master Thesis (Master in ExperimentalPathology)–Universidade Estadual de Londrina, Londrina, 2011.

## ABSTRACT

There are several therapies for neuropathic pain control, but current treatments are lacking efficacy and have many side effects, so alternatives for this treatment are needed. Fructose-1, 6-bisphosphate (FBP) is an intermediate of the glycolytic pathway that has several pharmacological actions, and more recently it was demonstrated its anti-hyperalgesic effect in acute inflammatory pain model via production of adenosine. Interestingly, adenosine and adenosine A1 and A2A receptor agonists have anti-hyperalgesic effects in neuropathic pain models. Thus, we investigated the anti-hyperalgesic effect and mechanism of action of FBP in a model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain in Swiss mice. Seven days after surgery, animals were treated with FBP or adenosine in oral or intrathecal administration. Moreover, the oral treatments of FBP and adenosine were associated with A1 or A2A adenosine receptor antagonists in intraplantar or intrathecal administration. Finaly, oral treatment of FBP was associated with inhibitors of nitric oxide/cycle GMP/Protein Kinase G/Potassium Channels ATP-sensitive (NO/cGMP/PKG/KATP) signaling pathway, which was already demonstrated as mechanism of action of adenosine. The mechanical hyperalgesia was assessed by an electronic version of von Frey filaments 1, 3, 5 and 7 hours after treatments. FBP and adenosine inhibited the mechanical hyperalgesia induced by CCI in a similar profile. FBP mechanism of action seems to be dependent on adenosine production as its effect was inhibited by adenosine A1 and A2A receptors antagonists, and FBP treatment promote anti-hyperalgesia through NO/cGMP/PKG/KATP signaling pathway similarly to adenosine. Concluding, it was demonstrated that FBP has possible therapeutic application to reduce neuropathic pain, and its mechanisms involve adenosine activation of A1 and A2A receptors and the NO/cGMP/PKG/KATP signaling pathway.

**Keywords:** Hyperalgesia. Neuropathic pain. Fructose-1, 6-bisphosphate. Adenosine. Adenosine receptors. Nitric oxide.

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# 1 INTRODUÇÃO

## 1.1 DOR E NOCICEPÇÃO

A dor é definida pela Associação Internacional para Estudo da Dor (IASP) como sendo “experiência sensorial e emocional desagradável associada a dano tecidual potencial ou real” (Merskey e Bodduk, 1994). Portanto, a dor é uma experiência complexa e subjetiva, formada por componente sensorial-discriminativo, que permite identificar o estímulo como doloroso e localizar a origem da lesão, e componente emocional-motivacional, que envolve a aprendizagem, memória e as reações da dor (Calvino e Grilo, 2005). A habilidade de detectar estímulos potencialmente destrutivos ou dolorosos (nociceptivos) pelo sistema somatossensorial é um mecanismo de proteção muito importante que envolve a interação de vários mecanismos periféricos e centrais. A detecção de estímulos dolorosos depende da ativação dos nociceptores e da transmissão desta informação ao SNC.

Se o dano tecidual for inevitável, uma gama de alterações na excitabilidade no SNC (sistema nervoso central) e SNP (sistema nervoso periférico) se estabelecem com um profundo, mas reversível, estado de dor e hipersensibilidade no tecido inflamado e nas suas adjacências. Esse processo facilita a reparação das partes lesadas, evitando o contato local até que a cura aconteça.

A nocicepção (do latim *nocere*, “nocivo”), termo empregado inicialmente por Sherrington (1906), é a detecção seletiva de estímulos capazes de comprometer a integridade física de um organismo. Estudos comportamentais com modelos humanos e animais utilizam os termos *alodinia* e *hiperalgesia*, sendo o primeiro utilizado para descrever sensações de dor induzida por estímulos mecânicos ou térmicos inócuos, enquanto que a hiperalgesia refere-se ao aumento exacerbado da resposta a um estímulo doloroso.

O estímulo doloroso (ou nociceptivo), seja ele físico (mecânico ou térmico) ou químico (bradicinina, histamina, serotonina, potássio, acetilcolina), é detectado por nociceptores, que são terminações nervosas livres, de fibras aferentes primárias. As fibras aferentes primárias são classificadas em fibras amplamente mielinizadas – Aβ –, que transmitem informações como toque leve ou estímulos

mecânicos inócuos para estruturas no corno dorsal da medula espinal, nas lâminas III e IV (Cousins e Cohen, 2005), fibras finamente mielinizadas – as Aδ –, com velocidade de condução de 5-30 m/s, e em fibras não mielinizadas – fibras C –, com velocidade de condução menor que 1,0 m/s.

As fibras C respondem a estímulos dolorosos de origem térmica, mecânica ou química e, por isso, são chamadas de polimodais. Já as fibras Aδ, respondem a estímulos dolorosos térmicos e mecânicos (Julius e Basbaum, 2001). Além disso, existe a classe de nociceptores, denominados silenciosos, descritos por Schaible e Schimidt (1988), que fisiologicamente não respondem a estímulos mecânicos ou térmicos, mas são ativados durante processos inflamatórios.

A ativação do aferente primário resulta da liberação de mediadores excitatórios, levando diferentes informações quanto à localização e intensidade do estímulo doloroso ao SNC por diferentes vias ascendentes (Loeser e Melzack, 1999).

A informação nociceptiva inicia-se pela ativação do nociceptor, gerando um sinal elétrico, que é conduzido por toda extensão da fibra aferente primária, até alcançar variados níveis da medula espinal. Nestes locais, as fibras aferentes primárias promovem sinapses com neurônios de segunda ordem, cujos axônios principais, em conjunto, formam os tratos ou vias ascendentes que conduzem a informação nociceptiva para diversas regiões supraespinais, dentre elas o tálamo e o córtex somatossensorial (Willis, 1985), onde será interpretada como dor (Noback, Strominger e Demarest, 1996).

Os diferentes tratos já descritos na literatura compõem dois sistemas ascendentes: 1) o sistema que trafega medialmente pelo tronco cerebral, composto pelos tratos denominados paleoespinotalâmico, espinorreticular, espinomesencefálico e proprioespinal; e 2) o sistema que mantém curso lateral pelo tronco cerebral e conduz rapidamente a dor, composto pelos tratos neoespinotalâmico, espinocervical e dos núcleos da coluna dorsal (Prado, 1999). No caminho oposto, também existem vias descendentes que modulam a transmissão da dor na medula espinal por ações pré-sinápticas em fibras aferentes primárias, ações pós-sinápticas em projeções neuronais, ou afetando interneurônios no corno dorsal da medula espinhal (Cousins; Cohen, 2005).

## 1.2 DOR NEUROPÁTICA

A dor neuropática é definida pela IASP como dor causada por uma lesão ou disfunção do sistema nervoso somatossensorial. Dependendo do local da lesão, a dor neuropática pode ser classificada em central ou periférica.

As causas da dor neuropática periférica incluem as lesões traumáticas de nervos, doenças vasculares (isquemias), metabólicas *(diabetes mellitus)*, inflamatórias (lúpus eritematoso sistêmico), infecciosas (HIV), intoxicação por metais pesados, deficiência de vitamina (B12), compressão de nervo, raízes e plexos nervosos, amputações (dor fantasma) e câncer (Souza, 2005).

O dano ou disfunção neural pode manifestar-se por sinais sensoriais negativos como anestesia ou hipoestesia e por sinais sensoriais positivos como dor espontânea (estímulo independente), hiperalgesia e alodinia (Dworkin et al., 2003; Chong e Bajwa, 2003).

Dentre as diversas condições em que ocorrem estados dolorosos, os quadros de neuropatia estão entre os mais desafiadores quanto ao tratamento, pois são, frequentemente, resistentes aos analgésicos conhecidos e comprometem a qualidade de vida de milhões de pessoas no mundo.

Durante a última década, evidências acumuladas sobre como lesão do nervo periférico cria dor neuropática sugerem que a lesão do nervo produz alterações moleculares e celulares, que resultam em múltiplas formas de plasticidade neuronal e reorganizações anatômicas (Inoue et al., 2007).

O processo pelo qual a lesão de um nervo periférico desencadeia dor envolve várias etapas onde a degeneração Walleriana desencadeia a atividade ectópica que leva a uma sensibilização central.

A degeneração Walleriana faz parte da reação de células da glia e imunológicas à lesão do nervo, onde células de Schwann e macrófagos passam a fagocitar a porção distal do nervo periférico lesionado, liberando uma série de substâncias no local da lesão, que sensibilizam os nociceptores e atraem mais células para o local. Uma resposta imunológica no gânglio da raiz dorsal também ocorre e é desencadeada por macrófagos, linfócitos e células satélites. Finalmente, ocorre a ativação da micróglia espinhal que é seguida pela ativação e proliferação de astrócitos, no corno posterior da medula (Scholz e Woolf, 2007).

A atividade ectópica, que é o disparo de potenciais de ação ao longo do aferente primário, gânglio da raiz dorsal e raiz dorsal, está associada a desregulação dos canais de sódio (especialmente os canais resistentes a tetrodotoxina) e é desencadeada pelas interações neuroimunológicas.

A hiperexcitabilidade periférica, desencadeada pelos potenciais ectópicos, leva a modificações centrais, que são caracterizadas pela modificação dos controles modulatórios da transmissão de mensagens nociceptivas, pela reorganização anatômica central dos neurônios aferentes (neuroplasticidade) e sua ativação descontrolada, levando a sensibilização central. A sensibilização central depende de mudanças intracelulares que são induzidas pela ativação de receptores N-metil-D-aspartato (NMDA) por amino-ácidos excitatórios liberados pelos aferentes primários (Attal e Bouhassira, 1999).

Outra alteração responsável pela dor neuropática é a diminuição da neurotransmissão inibitória na medula espinhal, especialmente GABAérgica (Woof, 2004).

Devido a essa multiplicidade de mecanismos desencadeantes, a dor neuropática é muito complexa e difícil de ser tratada, assim, novas alternativas de tratamento precisam ser pesquisadas.

## 1.3 FRUTOSE-1, 6-BIFOSFATO

A mais de duas décadas Dr. Angel K. Markov demonstrou que a administração exógena de Frutose-1, 6-bifosfato (FBP) promove proteção contra danos decorrentes da hipóxia isquêmica. A teoria proposta foi que a administração de FBP levaria a uma manutenção do metabolismo: “Frutose-1, 6-bifosfato restaurará a atividade da glicólise, previamente inibida pela acidez, por intervir na via de Embden-Meyerhoff como um regulador metabólico e como um substrato de alta energia”. Ao invés do substrato, a entrada de uma molécula exógena de FBP na glicólise intracelular pouparia a despesa de dois ATPs (um pela hexoquinase e outro pela fosfofrutoquinase). Assim, a proposta original era que o metabolismo de FBP forneceria duas vezes mais rendimento que o da energia glicolítica, quatro ATPs por molécula de substrato (Markov, 1986).

A FBP é uma substância composta por um monossacarídeo frutose fosforilado nos carbonos C-1 e C-6, com atividade óptica dextrógira. Intermediário

altamente energético da via glicolítica, a FBP é portadora de diversos efeitos farmacológicos, incluindo: inibição da aderência leucocitária e disfunção microvascular no músculo esquelético em modelos de isquemia e reperfusão (Akimitsu et al., 1995); redução de edema de patas induzido por carragenina (Planas et al., 1993); redução dos indicadores de pleuresia (Alves-Filho et al., 2004); diminuição da produção intracelular de espécies reativas de oxigênio (ROS) e de prostaglandinas – E2 (Gámez et al., 2008; Ahn et al., 2002), aumento dos níveis de glutationa reduzida (Vexler et al., 2003) e diminuição da produção intracelular de citocinas inflamátorias como o TNF-alfa (Cuesta et al., 2006; Lopes et al., 2006; Yin et al., 2008).

A FBP apresenta também atividade imunomoduladora como redução da proliferação de células T *in vitro* (Bordignon-Nunes et al. 2003; Lopes et al., 2006), atividade anti-convulsivante (Lian et al., 2007 e 2008; Ding et al., 2010), prevenção de hepatite induzida por galactosamina (de Oliveira et al., 1992; Calafell et al., 2009), preservação de fígado em transplantes (Mihas et al., 2003; Moresco et al., 2004), atividade citoprotetora em lesões tóxicas no cérebro (Song et al., 2005), coração (Gawarammanaet al., 2010) e rins (Azambujaet al., 2011) e melhoria no quadro de sepse (Nunes et al., 2003; de Oliveira et al., 2010). Além disso, a FBP têm sido constantemente relatada na literatura por trabalhos em que sua aplicação terapêutica aponta atenuação significativa de injúrias em modelos experimentais de isquemias e reperfusões em rins (Didlake et al., 1989), fígado (Nakai et al., 1991), intestino (Sun et al., 1990; Sola et al., 2003), cérebro (Farias et al., 1990; Kaakinen et al., 2006), miocárdio (Riedel et al., 2004) e pulmões (Chu et al., 2002). Um único trabalho na literatura relatou efeito antinociceptivo em modelo de inflamação aguda (Valério et al., 2009).

Hardin et al. (2001) salientaram que embora a FBP seja um agente terapêutico promissor em diversos modelos de doença, pouco tem sido feito para elucidar seu mecanismo de ação.

Não obstante a FBP ser uma molécula muito polar e altamente carregada no pH fisiológico, já está bem sedimentado na literatura sua capacidade de atravessar a membrana celular, inclusive de maneira dose-dependente (Ehringer et al., 2000). Embora o mecanismo de seu transporte não esteja esclarecido e um transportador próprio de membrana não tenha sido identificado, Hardin e Roberts (1994) usando carbono-13 em ressonância magnética nuclear, revelam uma

evidência direta que FBP exógena marcada com carbono-13 é capaz de atravessar a membrana plasmática, demonstrando, de fato, que a FBP possui essa capacidade. A literatura sugere quatro mecanismos para esclarecer essa passagem da FBP para o interior celular: (i) FBP usufrui de um carreador de membranas ainda não identificado; (ii) FBP se ligaria a uma forma lipídica solúvel de adolase que promoveria seu acesso; (iii) endocitose de fluido extracelular contendo FBP; (iv) permeabilidade passiva através da membrana plasmática (Galzingna et al., 1989; Hardin e Roberts, 1994; Rigobello et al., 1982).

Neste contexto, a FBP apresentou-se, em determinadas condições, capaz de aumentar o fluxo glicolítico e atuar como substrato para essa cascata reacional, evidenciando sua capacidade de atingir o citosol em quantidade suficiente para interferir com a via glicolítica (Hassinen et al., 1991; Lazzarino et al., 1991; Nuutinen et al., 1991). Em decorrência disso, quando a FBP entrar na célula, a via glicolítica não poderá ser significativamente regulada porque a FBP situa-se após as enzimas hexoquinase e fosfofrutoquinase – pontos de regulação da via – e antes da piruvatoquinase (terceiro ponto de regulação da via glicolítica) sobre a qual, todavia, a FBP atua como ativador alostérico (Bailey et al. 1968; Irving e William 1973), logo a produção de ATP será estimulada pois a piruvatoquinase não será um oponente à sua metabolização. Ainda, além de atuar como substrato para a via glicolítica e, consequentemente, elevar a produção de ATP. A FBP também pode elevar os níveis de ATP ao facilitar a ressíntese de ATP a partir de ADP (Loguercio et al., 1996).

## 1.4 ADENOSINA

Adenosina é um nucleosídeo purínico que está amplamente distribuído pelo corpo, classificada como neuromodulador endógeno e agonista purinérgico, produzida bioquimicamente através da união de uma base púrica adenina com uma D-ribose, é um dos maiores ribonucleotídeos do organismo (Sawynok, 1999).

Sob condições normais ela é continuamente formada tanto intracelularmente quanto extracelularmente (Fredholm, 2001), todavia em locais de dano tecidual é produzida em altas concentrações, desempenhando papel importante na regulação da homeostase de muitos sistemas fisiológicos, incluindo cardiovascular, nervoso, renal e imune (Blackburn, 2003). É liberada por várias

células como fibroblastos, células epiteliais e endoteliais, plaquetas e células musculares, embora também possa ser derivada do metabolismo extracelular de nucleosídeos purínicos (Lappas, Rieger, Linden, 2005).

Além disso, em condições metabolicamente desfavoráveis, tais como hipóxia, isquemia ou inflamação, a adenosina atua como um potente mensageiro extracelular cuja formação está usualmente elevada devido a sua liberação por células pertencentes ou não ao sistema imune e pela rápida degradação de ATP e ADP em adenosina (Cronstein et al., 1985; Okusa, 2002; Thiel, Caldwell, Sitkovsky, 2003). A via para produção de adenosina envolve uma cascata de desfosforilação de ATP e ADP finalizada sob ação de enzimas do tipo 5'-nucleotidases localizadas tanto na membrana celular quanto no interior celular (Dunwiddie et al., 1997; Schubert et al., 1979; Zimmermann et al., 1998 e 2000). Assim, o metabólito adenosina é produzido principalmente pela quebra de nucleotídeos de adenina intra ou extracelular (Zimmermann, 2000).

A adenosina intracelularmente formada, então, é derivada do ATP submetido à atividade de enzimas de 5'-nucleotidases intracelulares, também citadas por 5'-endonucleotidase ou 5'-nucleotidase citoplasmática (Borowiec et al., 2006). Em condições fisiológicas, a maior parte da adenosina é derivada do AMP intracelular que se difunde a favor de um gradiente de concentração para o exterior celular e encontra a ecto-5'-nucleotidase ancorada na membrana celular, uma enzima responsável por converter AMP em adenosina, e então a desfosforilação de AMP para adenosina é considerada o último passo desta cadeia enzimática (Fredholm et al., 2001).

No meio extracelular, os nucleotídeos também podem originar adenosina pela hidrólise por um conjunto de 5'-nucleotidases que se localizam na superfície celular, no meio intersticial ou em fluidos corporais e por essa razão denominadas ectonucleotidases (Zimmernann et al., 1998).

Desse modo, as ectonucleotidases juntamente com as 5'-endonucleotidases controlam não só a disponibilidade de ligantes (ATP, ADP, AMP e adenosina) para receptores de nucleotídeos, como também a duração e extensão da ativação de receptores (Chen e Guidotti, 2001). A modulação de concentrações de adenosina extracelular, por exemplo, por inibidores do metabolismo de adenosina, pode produzir atividades farmacológicas como antinociceptiva e anti-inflamatória dentre outras (Millan, 2002; Sawynok et al., 1999), uma vez que a

adenosina modula diversos processos fisiológicos mediados por seus quatro receptores: A1, A2A, A2B, A3 (Klinger et al., 2002).

Esses receptores podem estimular ou inibir a atividade da enzima adenilato ciclase e aumentar ou diminuir, respectivamente, a produção de AMPc (Fredholm et al., 2007); já que os receptores A1 e A3 são acoplados a $G_i$ ou $G_0$ e os receptores A2A e A2B são acoplados a $G_s$ (Khasar et al., 1995). Dessa maneira, tal inibição ou estímulo da adenilato ciclase proporciona diminuição ou aumento, respectivamente, das concentrações do segundo mensageiro AMPc, inibindo ou estimulando as vias dependentes desta molécula sinalizadora (Sawinok et al., 1999).

A complexidade de distribuição destes receptores em diferentes células reflete os múltiplos papéis que a adenosina apresenta no organismo.

Os receptores de adenosina A1 são expressos principalmente em neurônios, localizados no cérebro, no corno posterior da medula e aferentes primários (Hasko et al., 2007). Já os receptores A2A localizam-se em praticamente todas as células que participam da resposta imunológica como macrófagos, monócitos, células dendríticas, mastócitos, neutrófilos, eusinófillos, linfócitos, células endoteliais e epiteliais (Hasko et al., 2008).

Os receptores A2B são considerados de baixa afinidade a adenosina e são encontrados no intestino e bexiga e distribuídos em células como fibroblastos, células endoteliais e epiteliais (Cagnina et al., 2009), os receptores A3 estão em uma grande variedade de tecidos como testículos (Rivkees, 1994), cérebro, tecidos endócrinos e em células como os mastócitos (Baram et al., 2010).

Apesar dos tipos celulares envolvidos com a produção de adenosina extracelular não estarem totalmente esclarecidos, são conhecidos muitos tecidos que produzem adenosina como subproduto da degradação do ATP. Por exemplo, em neurônios e células da glia, a adenosina é liberada por meio de operação de sistemas de transporte da membrana quando a demanda se torna necessária (Sawinok e Sweeney, 1989; Sawinok, 1998, 1999). Nesse sentido, a adenosina extracelular pode ser ambiguamente gerada tanto em decorrência do uso do ATP como fonte de energia, quanto como neurotransmissor, sendo que em repouso ocorre a produção e manutenção de níveis basais de adenosina.

## 1.5 ADENOSINA E DOR NEUROPÁTICA

A administração exógena de adenosina demonstra efeito antinociceptivo em modelos animais de neuropatia (Balasubramanyan et al., 2008; Bantel et al 2002) e em humanos com dor neuropática (Eisenach et al., 2002 e 2003; Belfrage et al., 1995 e 1999; Lynch et al., 2003 Sjölund et al., 2001).

Alguns estudos têm explorado o efeito anti-hiperalgésico de agonistas do receptor A1, já que estes receptores são encontrados predominantemente em neurônios (Hasko et al., 2007). De fato, os agonistas dos receptores A1 tem efeito antinociceptivo em vários modelos de dor (Lee e Yaksh, 1996; Yamamoto et al., 2003; Zahn et al., 2007), incluindo dor neuropática (Cui et al., 1997; Curros-Criado et al., 2005; Gong et al., 2010).

Há evidências de ações inibitórias mediadas por receptores de adenosina A1 em terminais nervosos aferentes primários, em interneurônios e neurônios de projeção no corno posterior da medula espinhal (Santicioli et al., 1993; Lao et al., 2001; Patel et al., 2001).

O possível mecanismo de ação dos agonistas do receptor A1 na inibição da dor foi relatado por Lima et al. (2010); onde demonstraram que a ativação de dos receptores A1 leva a um aumento da produção de óxido nítrico que consequentemente ativa a guanilato ciclase, elevando assim os níveis de GMPc, ativando por consequência a proteína quinase G (PKG) que fosforila os canais de potássio ATP sensíveis aumentando assim o limiar de excitabilidade neuronal.

Essa cascata de sinalização intracelular foi evidenciada nos anos 1990 e é o mecanismo de ação de vários analgésicos como dipirona e morfina. (Sachs et al., 2003; Cunha et al., 2010; Cury et al., 2011).

Um crescente número de evidências mostra que agonistas do receptor A2A têm efeito anti-inflamatório e imunorregulatório potentes sobre células imunes, incluindo a supressão da produção de citocinas pró-inflamatórias e aumento da produção de citocinas anti-inflamatórias como a IL-10. Considerando que a indução da dor neuropática depende de interações neuroimunes, o uso deste agonista poderia ter efeito antinociceptivo neste caso; o que de fato, foi demonstrado por Loram et al. (2009) que mostraram que a injeção intratecal de agonista do receptor A2A reverteu a dor neuropática e este efeito envolveria, provavelmente, a

supressão de astrócitos, que são células com propriedades imunogênicas, e inibição da ativação da microglia, que são os macrófagos do SNC (Ren e Dubner, 2008).

Ainda há evidências, em outros modelos de dor, que a administração sistêmica de antagonista dos receptores A2A reduz o efeito antinociceptivo da inosina (um metabólito da adenosina) (Nascimento et al., 2010) e que um agonista do receptor A2A induz analgesia no teste da formalina (Borghi et al., 2002).

Por outro lado, há evidências de que a ativação do receptor A2A pode ser pró-nociceptiva, especialmente quando agonistas deste receptor são administrados perifericamente (Taiwo e Levine, 1990; Doak e Sawynok, 1995; Khasar et al, 1995). Além disso, camundongos knockout para o receptor A2A demonstram quadro de hipoalgesia para dor aguda (Ledent et al., 1997) e dor neuropática (Bura et al., 2008).

## 1.6 Relação Entre Frutose-1, 6-Bifosfato e Adenosina

Sola et al. (2003) apresentaram evidências farmacológicas de que certas propriedades anti-inflamatórias da FBP poderiam estar relacionadas a alterações dos níveis endógenos de adenosina, uma vez que as atividades da FBP descritas pelos autores foram inibidas pela administração de uma enzima metabolizadora de adenosina, a adenosina deaminase (ADA).

Recentemente, Valério et al. (2009) forneceram mais evidências de que a adenosina é crucial para o efeito farmacológico de FBP, uma vez que o tratamento com antagonista do receptor A1 inibiu o efeito antinociceptivo da FBP e além disso, que a administração da FBP aumenta os níveis de adenosina no sangue constado por HPLC.

Em seu trabalho Valério et al. (2009) propôs a seguinte explicação para o aumento de adenosina em função da administração exógena de FBP: a FBP entra nas células de maneira ainda não identificada (Hardin e Roberts, 1994), no meio intracelular, a FBP desregula a via glicolítica por estar a frente de duas enzimas reguladoras da via (hexoquinase e fosfofrutoquinase) e depois da piruvato quinase da qual é ativador alostérico (Taylor e Bailey, 1967; Bailey et al., 1968; Irving e Williams, 1973). Portanto, é possível que a FBP cause um aumento nos intermediários de glicólise, induzindo um efluxo de AMP para fora da célula. Este

AMP poderia ser hidrolisado à adenosina no meio extracelular que estaria, então, disponível para se ligar a seus receptores.

Desta maneira, é possível que parte dos benefícios farmacológicos da FBP se deva a produção de adenosina, mas isso precisa ser melhor investigado.

## BIBLIOGRAFIA

Ahn, S.M., Yoon, H.Y., Lee, B.G., Park, K.C., Chung, J.H., Moon, C.H., Lee, S.H., 2002. Fructose-1,6-diphosphate attenuates prostaglandin E2 production and cyclo-oxygenase-2 expression in UVB-irradiated HaCaT keratinocytes. Br. J. Pharmacol. 137, 497-503.

Akimitsu, T., White, J.A., Carden, D.L., Gute, D.C., Korthuis, R.J., 1995. Fructose-1,6-diphosphate or adenosine attenuate leukocyte adherence in postischemic skeletal muscle. Am. J. Physiol. 269, 1743-51.

Alves Filho, J.C., Santos, R.C., Castaman, T.A., de Oliveira, J.R., 2004. Anti-inflammatory effects of fructose-1,6-bisphosphate on carrageenan-induced pleurisy in rat. Pharmacol.Res. 49(3), 245-8.

Attal, N., Bouhassira, D., 1999. Mechanisms of pain in peripheral neuropathy. Acta. Neurol. Scand. Suppl. 173, 12-24.

Azambuja, A.A., Lunardelli, A., Nunes, F.B., Gaspareto, P.B., Donadio, M.V., de Figueiredo, C.E., de Oliveira, J.R., 2011. Effect of fructose-1,6-bisphosphate on the nephrotoxicity induced by cisplatin in rats. Inflammation 34(1), 67-71.

Bailey, E., Stirpe, F., Taylor, C.B., 1968. Regulation of rat liver pyruvate kinase. The effect of preincubation, pH, copper ions, fructose-1,6-diphosphate and dietary changes on enzyme activity. Biochem. J. 108(3), 427-36.

Balasubramanyan, S., Sharma, S.S., 2008. Protective effect of adenosine in diabetic neuropathic pain is mediated through adenosine A1-receptors. Indian J. Physiol. Pharmacol. 52(3), 233-42.

Bantel, C., Tobin, J.R., Li, X., Childers, S.R., Chen, S.R., Eisenach, J.C., 2002. Intrathecal adenosine following spinal nerve ligation in rat: short residence time in cerebrospinal fluid and no change in A(1) receptor binding. Anesthesiol. 96(1), 103-8.
Baram, D., Dekel, O., Mekori, Y.A., Sagi-Eisenberg, R., 2010. Activation of mast cells by trimeric G protein Gi3; coupling to the A3 adenosine receptor directly and upon T cell contact. J. Immunol. 184(7), 3677-88.

Belfrage, M.,Segerdahl, M., Arnér, S., Sollevi, A., 1999. The safety and efficacy of intrathecal adenosine in patients with chronic neuropathic pain. Anesth. Analg. 89(1), 136-142.

Belfrage, M.,Sollevi, A., Segerdahl, M., Sjölund, K.F., Hansson, P., 1995. Systemic adenosine infusion alleviates spontaneous and stimulus evoked pain in patients with peripheral neuropathic pain. Anesth. Analg. 81(4),713-117.

Blackburn, M.R., 2003. Too much of a good thing: adenosine overload in adenosine-deaminase-deficient mice. Trends Pharmacol. Sci. 24(2), 66-70.

Bordignon Nunes, F., Meier Graziottin, C., Alves Filho, J.C., Lunardelli, A., Caberlon, E., Peres, A., Rodrigues De Oliveira, J., 2003. Immunomodulatory effect of fructose-1,6-bisphosphate on T-lymphocytes. Int. Immunopharmacol. 3(2), 267-72.

Borghi, V., Przewlocka, B., Labuz, D., Maj, M., Ilona, O., Pavone, F., 2002. Formalin-induced pain and mu-opioid receptor density in brain and spinal cord are modulated by A1 and A2a adenosine agonists in mice. Brain Res. 956(2), 339-48.

Borowiec, A., Lechward, K., Tkacz-Stachowska, K., Składanowski, A.C., 2006. Adenosine as a metabolic regulator of tissue function: production of adenosine by cytoplasmic 5'-nucleotidases. Acta. Biochim. Pol. 53(2), 269-78.

Bura, S.A., Nadal, X., Ledent, C., Maldonado, R., Valverde, O., 2008. A2A adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. Pain 140(1), 95-103.

Cagnina, R.E., Ramos, S.I., Marshall, M. A., Wang, G., Frazier, C.R., Linden, J. 2009. Adenosine A2B receptors are highly expressed on murine type II alveolar epithelial cells. Am. J. Physiol. Lung Cell Mol. Physiol. 297(3), L467-74.

Calafell, R., Boada, J., Santidrian, A.F., Gil, J., Roig, T., Perales, J.C., Bermudez, J., 2009. Fructose 1,6-bisphosphate reduced TNF-alpha-induced apoptosis in galactosamine sensitized rat hepatocytes through activation of nitric oxide and cGMP production. Eur. J. Pharmacol. 610(1-3), 128-33.

Calvino, B., Grilo, R.M., 2006. Central pain control. Joint Bone Spine 73, 10-6.

Chen, W., Guidotti, G., 2001. Soluble apyrases release ADP during ATP hydrolysis. Biochem. Biophys. Res. Commun. 282(1), 90-5.

Chong, M.S., Bajwa, Z.H., 2003. Diagnosis and treatment of neuropathic pain. J. Pain Symptom Manage 25(5), S4-S11.

Cousins, M.J., Cohen, M.L., 2005. Physiology and Psychology of Acute Pain. In: Cousins MJ, Cohen ML (eds.). Acute Pain Management: Scientific Evidence. Melbourn, Australian and New Zealand College of Anaesthetists. 1-19.

Cronstein, B.N., Rosenstein, E.D., Kramer, S.B., Weissmann, G., Hirschhorn, R., 1985. Adenosine; a physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils. J. Immunol. 135(2), 1366-71.

Cuesta, E., Boada, J., Calafell, R., Perales, J.C., Roig, T., Bermudez, J., 2006. Fructose-1,6-bisphosphate prevented endotoxemia, macrophage activation, and liver injury induced by D-galactosamine in rats. Crit. Care Med. 34(3), 807-14.

Cui, J.G., Sollevi, A., Linderoth, B., Meyerson, B.A., 1997. Adenosine receptor activation suppresses tactile hypersensitivity and potentiates spinal cord stimulation in mononeuropathic rats. Neurosci. Lett. 223(3), 173-6.

Cunha, T.M., Roman-Campos, D., Lotufo, C.M., Duarte, H.L., Souza, G.R., Verri, W.A.Jr., Funez, M.I., Dias, Q.M., Schivo, I.R., Domingues, A.C., Sachs, D., Chiavegatto, S., Teixeira, M.M., Hothersall, J.S., Cruz, J.S., Cunha, F.Q., Ferreira, S.H., 2010. Morphine peripheral analgesia depends on activation of the PI3Kgamma/AKT/nNOS/NO/KATP signaling pathway. Proc. Natl. Acad. Sci. U.S.A. 107(9), 4442-7.

Curros-Criado, M.M., Herrero, J.F., 2005. The antinociceptive effects of the systemic adenosine A1 receptor agonist CPA in the absence and in the presence of spinal cord sensitization. Pharmacol. Biochem. Behav. 82(4), 721-6.

Cury, Y., Picolo, G., Gutierrez, V.P., Ferreira, S.R., 2011. Pain and analgesia: The dual effect of nitric oxide in nociceptive system. Nitric Oxide 25, 243-54.

De Oliveira, J.R., Rosa, J.L., Ambrosio, S., Bartrons, R., 1992. Effect of galactosamine on hepatic metabolism: protective role of fructose-1,6-bisphosphate. Hepatol. 15(6), 1147-53.

De Oliveira, L.M., Pires, M.G., Magrisso, A.B., Munhoz, T.P., Roesler, R., de Oliveira, J.R., 2010. Fructose-1,6-bisphosphate inhibits in vitro and ex vivo platelet aggregation induced by ADP and ameliorates coagulation alterations in experimental sepsis in rats. J. Thromb. Thrombolysis 29(4), 387-94.

Ding, Y.,Wang, S., Zhang, M.M., Guo, Y., Yang, Y., Weng, S.Q., Wu, J.M., Qiu, X., Ding, M.P., 2010. Fructose-1,6-diphosphate inhibits seizure acquisition in fast hippocampal kindling. Neurosci. Lett. 477(1), 33-6.

Doak, G.J., Sawynok, J., 1995. Complex role of peripheral adenosine in the genesis of the response to subcutaneous formalin in the rat. Eur. J. Pharmacol. 281, 311–318.

Dunwiddie, T.V., Diao, L., Proctor, WR., 1997. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. J. Neurosci. 17(20), 7673-82.

Dworkin, R.H. Backonja, M., Rowbotham, M.C., Allen, R.R., Argoff, C.R., Bennett, G.J., Bushnell,M.C., Farrar, J.T., Galer, B.S., Haythornthwaite, J.A., Hewitt, D.J., Loeser, J.D., Max, M.B., Saltarelli, M., Schmader, K.E., Stein, C., Thompson, D., Turk, D.C., Wallace, M.S, Watkins, L.R., Weinstein, S.M., 2003. Advances in neuropathic pain. Arch. Neurol. 60(11), 1524-1534.

Ehringer, W.D., Niu, W., Chiang, B., Wang, O.L., Gordon, L., Chien, S., 2000. Membrane permeability of fructose-1,6-diphosphate in lipid vesicles and endothelial cells. Mol. Cell Biochem. 210(1-2), 35-45.

Eisenach, J.C., Curry, R., Hood, D.D., 2002. Dose response of intrathecal adenosine in experimental pain and allodynia. Anesthesiol. 97(4), 938-942.

Eisenach, J.C., Rauck, R.L., Curry, R., 2003. Intrathecal, but not intravenous adenosine reduces allodynia in patients with neuropathic pain. Pain 105(1-2), 65-70.

Farias, L.A., Smith, E.E., Markov, A.K., 1990. Prevention of ischemic-hypoxic brain injury and death in rabbits with fructose-1,6-diphosphate. Stroke 21(4), 606-13.

Fredholm, B.B., Chern, Y., Franco, R., Sitkovsky, M., 2007. Aspects of the general biology of adenosine A2A signaling. Prog. Neurobiol. 83, 263–276.

Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

Gámez, A., Alva, N., Roig, T., Bermúdez, J., Carbonell, T., 2008. Beneficial effects of fructose-1,6-biphosphate on hypothermia-induced reactive oxygen species injury in rats. Eur. J. Pharmacol. 590(1-3), 115-9.

Gawarammana, I.,Mohamed, F., Bowe, S.J., Rathnathilake, A., Narangoda, S.K., Azher, S., Dawson, A.H., Buckley, N.A., 2010. Fructose-1, 6-diphosphate (FDP) as a novel antidote for yellow oleander-induced cardiac toxicity: a randomized controlled double blind study. BMC Emerg. Med. 29,10-15.

Gong, Q.J.,Li, Y.Y., Xin, W.J., Wei, X.H., Cui, Y., Wang, J., Liu, Y., Liu, C.C., Li, Y.Y., Liu, X.G., 2010. Differential effects of adenosine A1 receptor on pain-related behavior in normal and nerve-injured rats. Brain Res. 1361, 23-30.

Hardin, C.D., Lazzarino, G., Tavazzi, B., Di Pierro, D., Roberts, T.M., Giardina, B., Rovetto, M.J., 2001. Myocardial metabolism of exogenous FDP is consistent with transport by a dicarboxylate transporter. Am. J. Physiol. Heart Circ. Physiol. 281(6), H2654-60.

Hardin, C.D., Roberts, T.M., 1994. Metabolism of exogenously applied fructose 1,6-bisphosphate in hypoxic vascular smooth muscle. Am. J. Physiol. 267(6 Pt 2), H2325-32.

Haskó, G., Linden, J., Cronstein, B., Pacher, P., 2008. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat. Rev. Drug Discov. 7(9), 759-70.

Haskó, G., Pacher, P., Deitch, E.A., Vizi, E.S., 2007. Shaping of monocyte and macrophage function by adenosine receptors. Pharmacol. Ther. 113, 264 –275.

Hassinen, I.E., Nuutinen, E.M., Ito, K., Nioka, S., Lazzarino, G., Giardina, B., Chance, B., 1991. Mechanism of the effect of exogenous fructose 1,6-bisphosphate on myocardial energy metabolism. Circulation 83(2), 584-93.

Inoue, K., Tsuda, M., Tozaki-Saitoh, H., 2007. Modification of neuropathic pain sensation through microglial ATP receptors. Purinergic Signal 3(4), 311-6.

Irving, M.G., Williams, J.F., 1973. Kinetic studies on the regulation of rabbit liver pyruvate kinase. Biochem. J. 131(2), 287-301.

Julius, D., Basbaum, A.I., 2001. Molecular mechanisms of nociception. Nature. 413, 203- 10.

Kaakinen, T.,Heikkinen, J., Dahlbacka, S., Alaoja, H., Laurila, P., Kiviluoma, K., Salomäki, T., Romsi, P., Tuominen, H., Biancari, F., Lepola, P., Nuutinen, M., Juvonen, T., 2006. Fructose-1,6-bisphosphate supports cerebral energy metabolism in pigs after ischemic brain injury caused by experimental particle embolization. Heart Surg. Forum 9(6), E828-35.

Khasar, S.G., Wang, J.F., Taiwo, Y.O., Heller, P.H., Green, P.G., Levine, J.D., 1995. Mu-opioid agonist enhancement of prostaglandin-induced hyperalgesia in the rat: a G-protein beta gamma subunit-mediated effect? Neurosc. 67, 189 –195.

Klinger, M., Freissmuth, M., Nanoff, C., 2002. Adenosine receptors: G protein-mediated signalling and the role of accessory proteins. Cell Signal 14(2), 99-108.

Lao, L.J., Kumamoto, E., Luo, C., Furue, H., Yoshimura, M., 2001. Adenosine inhibits excitatory transmission to substantia gelatinosa neurons of the adult rat spinal cord through the activation of presynaptic A(1) adenosine receptor. Pain 94(3), 315-24.

Lappas, C.M., Rieger, J.M., Linden, J., 2005. A2A adenosine receptor induction inhibits IFN-gamma production in murine CD4+ T cells. J. Immunol. 174(2), 1073-80.

Lazzarino, G., Nuutinen, M.E., Tavazzi, B., Cerroni, L., Di Pierro, D., Giardina, B., 1991. Preserving effect of fructose-1,6-bisphosphate on high-energy phosphate compounds during anoxia and reperfusion in isolated langendorff-perfused rat hearts. J. Mol. Cell Cardiol. 23(1), 13-23.

Ledent, C., Vaugeois, J.M., Schiffmann, S.N., Pedrazzini, T., El Yacoubi, M., Vanderhaeghen, J.J., Costentin, J., Heath, J.K., Vassart, G., Parmentier, M., 1997. Aggressiveness, hypoalgesia, and high blood pressure in mice lacking the adenosine A2a receptor. Nature 388, 674–678.

Lee, Y.W., Yaksh, T.L., 1996. Pharmacology of the spinal adenosine receptor which mediates the antiallodynic action of intrathecal adenosine agonists. J. Pharmacol. Exp. Ther. 277, 1642–1648.

Lian, X.Y., Khan, F.A., Stringer, J.L., 2007. Fructose-1,6-bisphosphate has anticonvulsant activity in models of acute seizures in adult rats. J. Neurosci. 27(44), 12007-11.

Lian, X.Y., Xu, K., Stringer, J.L., 2008. Oral administration of fructose-1,6-diphosphate has anticonvulsant activity. Neurosci. Lett. 446(2-3), 75-7.

Lima, F.O., Souza, G.R., Verri, W.A.Jr., Parada, C.A., Ferreira, S.H., Cunha, F.Q., Cunha, T.M., 2010. Direct blockade of inflammatory hypernociception by peripheral A1 adenosine receptors: involvement of the NO/cGMP/PKG/KATP signaling pathway. Pain 151(2), 506-15.

Loeser, J.D., Melzack, R., 1999. Pain: an overview. Lancet 353, 1607-9.

Loguercio, C., Nardone, G., Sicolo, P., Cuomo, R., Del Vecchio, C., Budillon, G., 1996. Intravenous load of fructose and fructose 1,6-diphosphate: effects on uricemia in patients with nonalcoholic liver disease. Am. J. Gastroenterol. 91(3), 559-64.

Lopes, R.P., Lunardelli, A., Preissler, T., Leite, C.E., Alves-Filho, J.C., Nunes, F.B., de Oliveira, J.R., Bauer, M.E., 2006. The effects of fructose-1,6-bisphosphate and dexamethasone on acute inflammation and T-cell proliferation. Inflamm. Res. 55(8), 354-8.

Loram, L.C., Harrison, J.A., Sloane, E.M., Hutchinson, M.R., Sholar, P., Taylor, F.R., Berkelhammer, D., Coats, B.D., Poole, S., Milligan, E.D., Maier, S.F., Rieger, J., Watkins, L.R., 2009. Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine 2A receptor agonists: a novel therapy for neuropathic pain. J. Neurosci. 29(44),14015-25.

Lynch, M.E.,Clark, A.J., Sawynok, J., 2003. Intravenous adenosine alleviates neuropathic pain: a double blind placebo controlled crossover trial using an enriched enrolment design. Pain 103(1-2), 111-117.

Markov, A.K., 1986. Hemodynamics and metabolic effects of fructose 1-6 diphosphate in ischemia and shock--experimental and clinical observations. Ann. Emerg. Med. 15(12), 1470-7.

Merskey, H., Bogduk, N., 1994. IASP Task Force on Taxonomy: Classification of Chronic Pain: Description of Chronic Pain Syndromes and Definition of Pain Terms. 2nd ed. Seattle: IASP Press.

Mihas, A.A., Kanji, V.K., Mihas, T.A., Joseph, R.M., Markov, A.K., Heuman, D.M., 2003. Fructose diphosphate attenuates the acetaminophen-induced liver injury in the rat evidence for involvement of nitric oxide. Res. Commun. Mol. Pathol. Pharmacol.114, 253-66.

Millan, M.J., 2002. Descending control of pain. Prog Neurobiol. 66(6), 355-474.

Moresco, R.N., Santos, R.C., Alves Filho, J.C., Cunha, A.A., Dos Reis, C., Reichel, C.L., De Oliveira, J.R., 2004. Protective effect of fructose-1,6-bisphosphate in the cold storage solution for liver preservation in rat hepatic transplantation. Transplant Proc. 36(5), 1261-4.

Nakai, T., Tanimura, H., Tabuse, K., Nagai, Y., Mori, K., Yamoto, H., 1991. Beneficial effects of fructose-1,6-diphosphate infusion on liver regeneration after ischemic liver injury. Gastroenterol. Jpn. 1991 26(5), 611-8.

Nascimento, F.P., Figueredo, S.M., Marcon, R., Martins, D.F., Macedo, S.J.Jr., Lima, D.A., Almeida, R.C., Ostroski, R.M., Rodrigues, A.L., Santos, A.R., 2010. Inosine reduces pain-related behavior in mice: involvement of adenosine A1 and A2A receptor subtypes and protein kinase C pathways. J. Pharmacol. Exp. Ther. 334(2), 590-8.

Noback, C.R., Strominger, N.L., Demarest, R.J., 1996.The human nervous system: structure and function. 5th ed. Baltimore: Williams & Wilkins.

Nunes, F.B., Graziottin, C.M., Alves Filho, J.C., Lunardelli, A., Pires, M.G., Wächter, P.H., De Oliveira, J.R., 2003. An assessment of fructose-1,6-bisphosphate as an antimicrobial and anti-inflammatory agent in sepsis. Pharmacol. Res. 47(1), 35-41.

Nuutinen, E.M., Lazzarino, G., Giardina, B., Hassinen, I.E., 1991. Effect of exogenous fructose-1,6-bisphosphate on glycolysis in the isolated perfused rat heart. Am. Heart J. 122(2), 523-7.

Okusa, M.D., 2002. A(2A) adenosine receptor: a novel therapeutic target in renal disease. Am. J. Physiol. Renal Physiol. 282(1), F10-8.

Patel, M.K., Pinnock, R.D., Lee, K., 2001. Adenosine exerts multiple effects in dorsal horn neurones of the adult rat spinal cord. Brain Res. 920(1-2), 19-26.

Planas, M.E., Sánchez, S., González, P., Rodrigues de Oliveira, J., Bartrons, R., 1993. Protective effect of fructose 1,6-bisphosphate against carrageenan-induced inflammation. Eur. J. Pharmacol. 237(2-3), 251-5.

Prado, W.A., 1999. Medicamentos Analgésicos de Ação Central. In: Graeff, F.G.; Guimaraes, F.S. (eds.). Fundamentos de Psicofarmacologia. São Paulo: Atheneu.

Ren, K., Dubner, R., 2008. Neuron-glia crosstalk gets serious: role in pain hypersensitivity. Curr. Opin. Anaesthesiol. 21, 570–579.

Riedel, B.J., Gal, J., Ellis, G., Marangos, P.J., Fox, A.W., Royston, D. 2004. Myocardial protection using fructose-1,6-diphosphate during coronary artery bypass graft surgery: a randomized, placebo-controlled clinical trial. Anesth. Analg. 98(1), 20-9.

Rigobello, M.P., Bianchi, M., Deana, R., Galzigna, L., 1982. Interaction of fructose-1,6-diphosphate with some cell membranes. Agressologie. 23(2), 63-6.

Rivkees, S. A., 1994. Localization and characterization of adenosine receptor expression in rat testis. Endocrinol. 135, 2307-2313.

Sachs, D., Cunha, F.Q., Ferreira, S.H., 2004. Peripheral analgesic blockade of hypernociception: activation of arginine/NO/cGMP/protein kinase G/ATP-sensitive K+ channel pathway. Proc. Natl. Acad. Sci. U.S.A. 101(10), 3680-5

Santicioli, P., Del Bianco, E., Maggi, C.A., 1993. Adenosine A1 receptors mediate the presynaptic inhibition of calcitonin gene-related peptide release by adenosine in the rat spinal cord. Eur. J. Pharmacol. 231(1), 139-42.

Sawynok, J., Reid, A., Liu, X.J., 1999. Acute paw oedema induced by local injection of adenosine A(1), A(2) and A(3) receptor agonists. Eur. J. Pharmacol. 386(2-3), 253-61.

Schaible, H.G., Schimidt, R.F., 1988. Time course of mechanosensitivity changes in articular afferents during a developing experimental arthritis. J. Neurophysiol. 60(6), 2180-95.

Scholz, J., Woolf, C.J., 2007. The neuropathic pain triad: Neurons, immune cells and glia. Nat. Neurosci. 10, 1361–1368.

Schubert, P., Komp, W., Kreutzberg, G.W., 1979. Correlation of 5'-nucleotidase activity and selective transneuronal transfer of adenosine in the hippocampus. Brain Res. 168(2), 419-24.

Sherrington, C.S., 1906. The integrative action of the nervous system. New York: Scribner.

Sjölund, K.F., Belfrage, M., Karlsten, R., Segerdahl, M., Arnér, S., Gordh, T., Solevi, A., 2001. Systemic adenosine infusion reduces the area of tactile allodynia in neuropathic pain following peripheral nerve injury: a multi-centre, placebo-controlled study. Eur. J. Pain 5(2), 199-207.

Sola, A., Panés, J., Xaus, C., Hotter, G., 2003. Fructose-1,6-biphosphate and nucleoside pool modifications prevent neutrophil accumulation in the reperfused intestine. J. Leukoc. Biol. 73(1), 74-81.

Song, X.,Wu, B., Takata, T., Wang, X., Oizumi, X.S., Akisaki, T., Yokono, K., Sakurai, T., 2005. Neuroprotective effect of D-fructose-1,6-bisphosphate against beta-amyloid induced neurotoxicity in rat hippocampal organotypic slice culture: involvement of PLC and MEK/ERK signaling pathways. Kobe J. Med. Sci. 51(5-6),73-83.

Souza, M.C., 2005. Epidemiologia. In: Drummond JP, Marquez JO. Dor Neuropática: Fisiologia, Clínica e Terapêutica. Curitiba: Evidence, 19-33.

Sun, J.X., Farias, L.A., Markov, A.K., 1990. Fructose 1-6 diphosphate prevents intestinal ischemic reperfusion injury and death in rats. Gastroenterol. 98(1), 117-26.

Taiwo, Y.O., Levine, J.D., 1990. Direct cutaneous hyperalgesia induced by adenosine. Neurosci. 38(3), 757-62.

Thiel, M., Caldwell, C.C., Sitkovsky, M.V., 2003. The critical role of adenosine A2A receptors in downregulation of inflammation and immunity in the pathogenesis of infectious diseases. Microbes Infect. 5, 515–526.

Valério, D.A., Ferreira, F.I., Cunha, T.M., Alves-Filho, J.C., Lima, F.O., De Oliveira, J.R., Ferreira, S.H., Cunha, F.Q., Queiroz, R.H., Verri, W.A.Jr., 2009. Fructose-1,6-bisphosphate reduces inflammatory pain-like behaviour in mice: role of adenosine acting on A1 receptors. Br. J. Pharmacol. 158(2), 558-68.

Vexler, Z.S., Wong, A., Francisco, C., Manabat, C., Christen, S., Täuber, M., Ferriero, D.M., Gregory, G., 2003. Fructose-1,6-bisphosphate preserves intracellular glutathione and protects cortical neurons against oxidative stress. Brain Res. 960(1-2), 90-8.

Willis, W.D., 1985. Nociceptive pathways: anatomy and physiology of nociceptive ascending pathways. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 308: 253-70.

Woolf, C.J., 2004. Dissecting out mechanisms responsible for peripheral neuropathic pain: implications for diagnosis and therapy. Life Sci. 74(21), 2605-10.

Yamamoto, S., Nakanishi, O., Matsui, T., Shinohara, N., Kinoshita, H., Lambert, C., Ishikawa, T., 2003. Intrathecal adenosine A1 receptor agonist attenuates hyperalgesia without inhibiting spinal glutamate release in the rat. Cell. Mol. Neurobiol. 23,175–185.

Yin, H., Jin, X.B., Gong, Q., Yang, H., Hu, L.Y., Gong, F.L., Zhu, J.Y., 2008. Fructose-1,6-diphosphate attenuates acute lung injury induced by lipopolysaccharide in mice. Int. Immunopharmacol. 8(13-14), 1842-7.

Zahn, P.K., Straub, H., Wenk, M., Pogatzki-Zahn, E.M., 2007. Adenosine A1 but not A2a receptor agonist reduces hyperalgesia caused by a surgical incision in rats: a pertussis toxin-sensitive G protein-dependent process. Anesthesiol. 107, 797-806.

Zimmermann, H., Braun, N., Kegel, B., Heine, P., 1998. New insights into molecular structure and function of ectonucleotidases in the nervous system. Neurochem. Int. 32(5-6), 421-5.

Zimmermann, H., 2000. Extracellular metabolism of ATP and other nucleotides. Naunyn Schmiedebergs Arch. Pharmacol. 362(4-5), 299-309.

# 2 OBJETIVOS

## 2.1 OBJETIVO GERAL

Investigar o efeito anti-hiperalgésico e mecanismo de ação da FBP em modelo de neuropatia periférica induzida pela constrição crônica do nervo ciático (CCI) em camundongos.

## 2.2 OBJETIVOS ESPECIFICOS

- Avaliar a curva resposta da FBP e adenosina na administração oral e intratecal no modelo de CCI
- Verificar a participação dos receptores de adenosina A1 e A2A no efeito terapêutico da adenosina e FBP no modelo de CCI;
- Verificar a participação da via de sinalização óxido nítrico/ GMPcíclico/proteína quinase G/canais de potássio ATP sensíveis (NO/GMPc/PKG/KATP) no efeito terapêutico da FBP no modelo de CCI.

# 3 ARTIGO

## Fructose-1, 6-Bisphosphate Reduces Neuropathic Pain Activating Adenosine A1 and A2A Receptors: Role of NO/cGMP/PKG/KATP Signaling Pathway

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**A ser submetido ao periódico Neuropharmacology**

# Fructose-1, 6-Bisphosphate Reduces Neuropathic Pain Activating Adenosine A1 and A2A Receptors: Role of NO/cGMP/PKG/KATP Signaling Pathway


## Abstract

There are several therapies for neuropathic pain control, but current treatments are lacking efficacy and have many side effects, so alternatives for this treatment are needed. Fructose-1, 6-bisphosphate (FBP) is an intermediate of the glycolytic pathway that has several pharmacological actions, and more recently it was demonstrated its anti-hyperalgesic effect in acute inflammatory pain model via production of adenosine. Interestingly, adenosine and adenosine A1 and A2A receptor agonists have anti-hyperalgesic effects in neuropathic pain models. Thus, we investigated the anti-hyperalgesic effect and mechanism of action of FBP in a model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain in swiss mice. Seven days after surgery, animals were treated with FBP, adenosine, A1 and A2A receptor antagonists and inhibitors of nitric oxide/cycle GMP/Protein Kinase G/Potassium Channels ATP-sensitive (NO/cGMP/PKG/KATP) signaling pathway, which was already demonstrated as mechanism of action of adenosine. The mechanical hyperalgesia was assessed by an electronic version of von Frey filaments 1, 3, 5 and 7 hours after treatment. FBP and adenosine inhibited the mechanical hyperalgesia induced by CCI in a similar profile. FBP mechanism of action seems to be dependent on adenosine production as its effect was inhibited by adenosine A1 and A2A receptors antagonists, and FBP treatment promote anti-hyperalgesia through NO/cGMP/PKG/KATP signaling pathway similarly to adenosine. Concluding, it was demonstrated that FBP has possible therapeutic application to reduce neuropathic pain, and its mechanisms involve adenosine activation of A1 and A2A receptors and the NO/cGMP/PKG/KATP signaling pathway.


**Keywords:** Hyperalgesia. Neuropathic Pain. Fructose-1, 6-bisphosphate. Adenosine. Adenosine Receptors. Nitric Oxide.

## 1. Introduction

Neuropathic pain is a major factor causing impaired quality of life in millions of people worldwide and is frequently resistant or poor analgesia is achieved with current analgesic drugs. Over the last decade, accumulating evidence concerning how peripheral nerve injury creates neuropathic pain has suggested that nerve injury produces molecular and cellular alterations that result in multiple forms of neuronal plasticity and anatomical reorganization which occurs in the site of the lesion, in dorsal root ganglia and in the dorsal horn of the spinal cord (Inoue et al.,2007 and Scholz and Woolf, 2007).

The process of peripheral nerve injury involves wallerian degeneration, ectopic activity and central sensitization and it is now clear that neurons are not the only players that drive the establishment and maintenance of this process that is manifested as pain. Recognition of the critical involvement of immune cells and glia in the pathophysiological changes after nerve injury offers new treatments approach (Watkins et al., 2007).

Fructose-1,6-bisphosphate (FBP) is a high-energy glycolytic pathway intermediate that has several pharmacological actions. The cytoprotective activity of FBP has been documented in severe conditions such as brain and myocardial ischemia (Kaakinen et al., 2006; Riedel et al., 2004), neurotoxic insults (Song et al., 2005), cardiac toxicity (Gawarammana et al., 2010) convulsions (Lian et al., 2008, Ding et al., 2010), reperfusion injury (Sola et al., 2003, Vexler et al., 2003), septic shock (de Oliveira et al., 2010), diabetic complications (Zhang et al., 2009; Xu et al., 2010), hypothermia-induced injury (Gámez et al., 2008), UV-provoked skin damage (Ahn et al., 2007), nephrotoxicity (Azambuja et al., 2011) and in other processes such apoptosis (Calafell et al., 2009).

Valério et al., 2009, showed for the first time that FBP has also anti-hyperalgesic effect in acute inflammatory pain model induced by carrageenan, and this effect was dependent on the production of adenosine, which in turn activated peripheral adenosine A1 receptors.

Curiously, adenosine and adenosine A1 receptor agonists have an anti-hyperalgesic effect in animal models of neuropathy (Balasubramanyan et al., 2008; Curros-Criado et al., 2005; Gong et al., 2010) and in humans with neuropathic pain (Eisenach et al., 2002 and 2003; Lynch et al., 2003; Belfrage et al., 1995 and 1999; Sjölund et al.,

2001). Moreover, Loran et al., 2009, showed the involvement of A2A adenosine receptor in the control of neuropathic pain.

A possible mechanism of action of FBP may be the activation of A1 receptor by adenosine, which leads to anti-hyperalgesia through NO/cGMP/PKG/KATP signaling pathway (Lima et al., 2010; Cury et al., 2011).

Considering the evidence that the FBP has anti-hyperalgesic effect in a model of inflammation (Valério DA et al., 2009) via production of adenosine and that adenosine reduces neuropathic pain, we propose to investigate the anti-hyperalgesic action and mechanisms of FBP in a model of neuropathy induced by chronic sciatic nerve constriction in mice.

## 2. Material and Methods

### 2.1. Animals

All animal care and experimental procedures were approved by the Ethics Committee of the Universidade Estadual de Londrina (CEUA-UEL/ 07219). Adult male Swiss mice (22–28 g) were obtained from the Universidade Estadual de Londrina and maintained in the department of Pathological Sciences of the Universidade Estadual de Londrina in a temperature-controlled room with access to water and food *ad libitum* until use.

### 2.2. Drugs

The agents used in this study and their sources were: Adenosine, DPCPX (1,3-dipropyl-8-cyclopentylxanthine), FBP, L-NMMA (NG-Monomethyl-L-arginine) and Glibenclamide from Sigma Chemical (St Louis, MO, USA). SCH442416 (2-(2-Furanyl)-7-[3-(4-methoxyphenyl)propyl]-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine) and ODQ (1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one) from Tocris Cookson (Ballwin, MO, USA). KT5823 ((9*S*,10*R*,12*R*)-2,3,9,10,11,12-Hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3',2',1'-*kl*]pyrrolo[3,4-*i*][1,6]benzodiazocine-10-carboxylic acid) from Calbiochem (San Diego, CA, USA). DPCPX was dissolved in Tween 80 0,5% in saline, Glibenclamide in Tween 80 2% in saline; ODQ and KT5823 were dissolved in DMSO 2% in saline.

SCH442416 was dissolved in DMSO 10% in saline and all the other drugs were dissolved in saline.

## 2.3. Chronic constriction injury (CCI)

Mice were operated under Bennett and Xie (1988) methods with minor modifications. The animals were anesthetized, the incision was made for access to the right sciatic nerve at the region of the upper femur. . One ligature with chromic suture was made in the sciatic nerve without nerve section, only constriction. Bennett and Xie (1988) described this procedure in rats using four ligatures. The increase in mechanical sensitivity in the paw remained significant for approximately 35 days after surgery. In sham animals (false-operated) the sciatic nerve was exposed but no ligated.

## 2.4. Mechanical hyperalgesia evaluation

Mechanical hyperalgesia was tested in mice as previously reported (Cunha et al., 2004). In a quiet room, mice were placed in acrylic cages (12 x10 x 17 cm) with wire grid floors, 15–30 min before the start of testing. The test consisted of evoking a hindpaw flexion reflex with a hand-held force transducer (electronic anaesthesiometer; Insight, Ribeirão Preto, SP, Brasil) adapted with a 0.5 $mm^2$ polypropylene tip. The investigator was trained to apply the tip perpendicularly to the central area of the hindpaw with a gradual increase in pressure. The end point was characterized by the removal of the paw followed by clear flinching movements. After the paw withdrawal, the intensity of the pressure was recorded automatically. The value for the response was an averaging of three measurements. The animals were tested before and after chronic sciatic nerve constriction and treatments. The results are expressed by delta (Δ) withdrawal threshold (in g) calculated by subtracting the zero-time mean measurements from the mean measurements.

## 2.5. Statistical Analysis

The results involving more than one variable such as treatment and time were analyzed using multivariate analysis of variance (MANOVA). When interaction was detected between time and treatment, further analysis was performed at each time

point using analysis of variance with one criterion (one-way ANOVA) followed by Bonferroni multiple comparisons t test. Differences were considered significant for $p<0.05$.

## 2.6. Experimental procedures

### *2.6.1. Evaluation of FBP dose-response effect*

Baseline mechanical hyperalgesia measures were recorded before the CCI or sham surgeries were conducted and were considered the zero-time measurements. Mechanical hyperalgesia evaluation was tested at 7 day after surgery. FBP or adenosine wereadministrated per oral (p.o.; 10, 30, 100, 300 mg/kg/100 µl).The groups were tested for mechanical hyperalgesia again at 1, 3, 5, 7 hours after administration of the drugs.

### *2.6.2. Effect of daily treatment with FBP and adenosine*

Between 7- 14 days after surgery, mice were treated p.o. with FBP, adenosine or vehicle (300 mg/kg/ 10 µl). The groups were tested for mechanical hyperalgesia again 3 hours after administration of the drugs, for 7 days.CCI surgery and behavioral measures were conducted as described in item 2.6.1.

### *2.6.3. Dose-response of anti-hyperalgesic effect of intrathecal administration of FBP or adenosine*

FBP or adenosine was administrated intrathecally (3, 10, 30 µg/animal/ 5 µl). CCI surgery and behavioral measures were conducted as described in item 2.6.1.

### *2.6.4. Adenosine A1 receptor antagonist effect in adenosine and FBP-induced anti-hyperalgesia*

DPCPX (adenosine A1 receptor antagonist) was administrated intrathecally (i.t.; 10, 3, 1 µg/animal/ 5 µl) and 30 minutes latter a dose of FBP or adenosine was administrated p.o. (300mg/Kg/100 µl). Afterwards, the dose of 10 µg/animal of DPCPX was chosen for intraplantar injection in FBP treated mice.CCI surgery and behavioral measures were conducted as described in item 2.6.1.

### *2.6.5. Adenosine A2 receptor antagonist effect in adenosine and FBP-induced anti-hyperalgesia*

SCH442416 (adenosine A2A receptor antagonist) was administrated i.t. (0,1, 0,3, 1 and 3 µg/animal/5 µl) and 30 minutes latter a dose of FBP or adenosine was administrated p.o. (300 mg/Kg/100 µl). Afterwards, the dose of 1 µg/animal of SCH442416 was chosen for intraplantar injection in FBP treated mice.CCI surgery and behavioral measures were conducted as described in item 2.6.1.

*2.6.6. Effect of inhibitors of nitric oxide activated signaling pathway in FBP and adenosine-induced anti-hyperalgesia.*

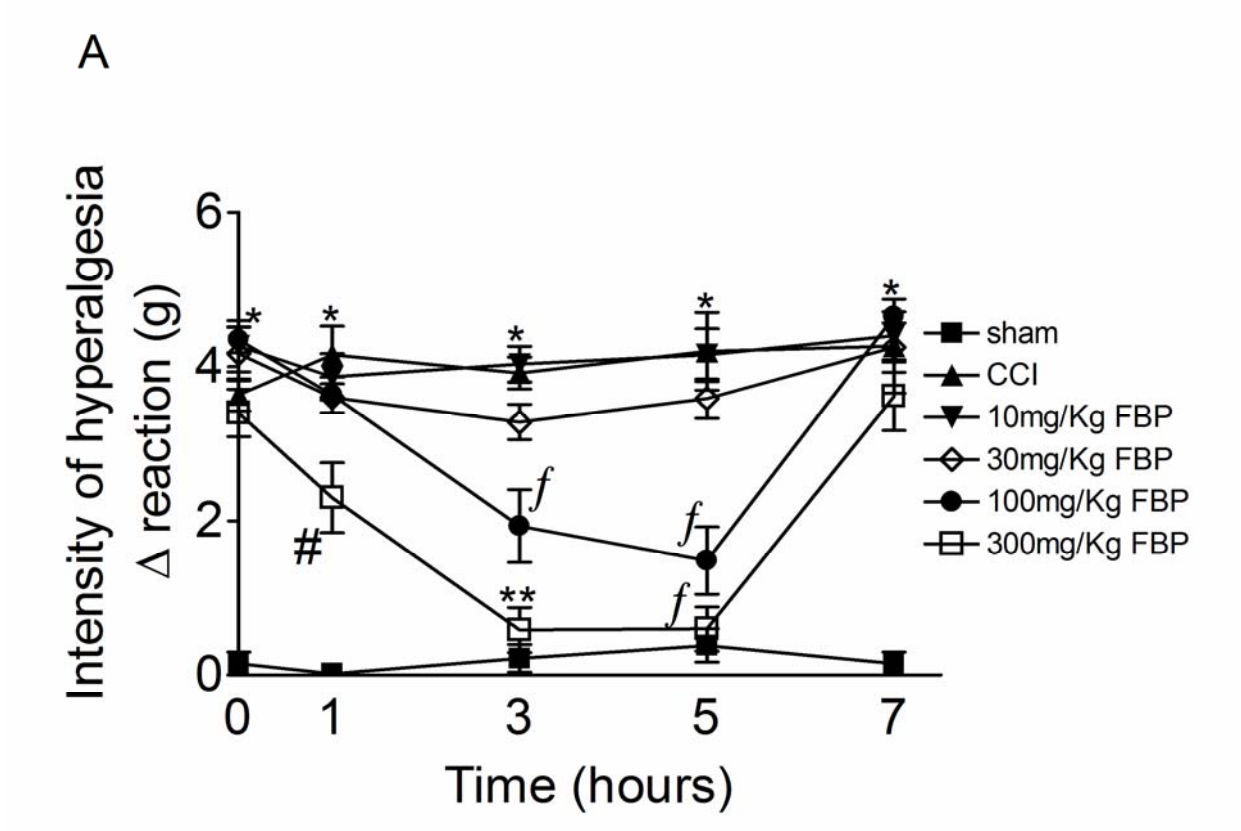
Mice were treated with i.p. with L-NMMA (non-selective inhibitor of neuronal nitric oxide synthase; 100 mg/Kg/100 µl, 45 min), ODQ (selective inhibitor of the soluble guanylyl cyclase, 1mg/Kg/100 µl, 30 min, Vale et al., 2007) or KT5823 (protein kinase G inhibitor, 0.5 µg/animal/100 µl, 5 min) or p.o. with glibenclamide (K+ATP channel blocker, 1mg/Kg/100 µl, 30 min, Vale et al., 2007) before FBP p.o. treatment (300 mg/Kg/100 µl). CCI surgery and behavioral measures were conducted as described in item 2.6.1.

## 3. Results

### 3.1. Dose-dependent anti-hyperalgesic effect of oral treatment with FBP and adenosine

Seven days after surgery, there was significant increase of mechanical hyperalgesia of CCI compared to sham-CCI mice (Fig. 1A). At 7 days, mice were treated p.o. with FBP (10-300mg/Kg) (Fig. 1A) or adenosine (10-300mg/kg) (Fig. 1B) and the intensity of hyperalgesia was measured before surgery, before treatments and 1-7 h after treatments.FBP and adenosine reduced the mechanical hyperalgesia induced by CCI in a dose-dependent manner. The doses of 10 and 30 mg/kg of FBP presented no statistical differences compared to the CCI control group (Fig. 1A). At 1h after treatment the dose of 300 mg/kg of FBP already present significant reduction of hyperalgesia compared to CCI group and the dose of 10 mg/kg of FBP. At 3 and 5h, the doses of 100 and 300 mg/kg of FBP presents significant reduction of hyperalgesia compared to CCI control group and the doses of 10 and 30 mg/kg of FBP. There was also significant difference between the doses of 100 and 300 mg/Kg 3 h after treatment. In adenosine treatments (Fig. 1B), there were significant

differences with all doses compared with the CCI group at 3 and 5 h. But the dose of 300 mg/Kg had significant difference from the CCI group since the first hour. Therefore, the p.o. dose of 300 mg/kg was used in subsequent experiments for FBP and adenosine.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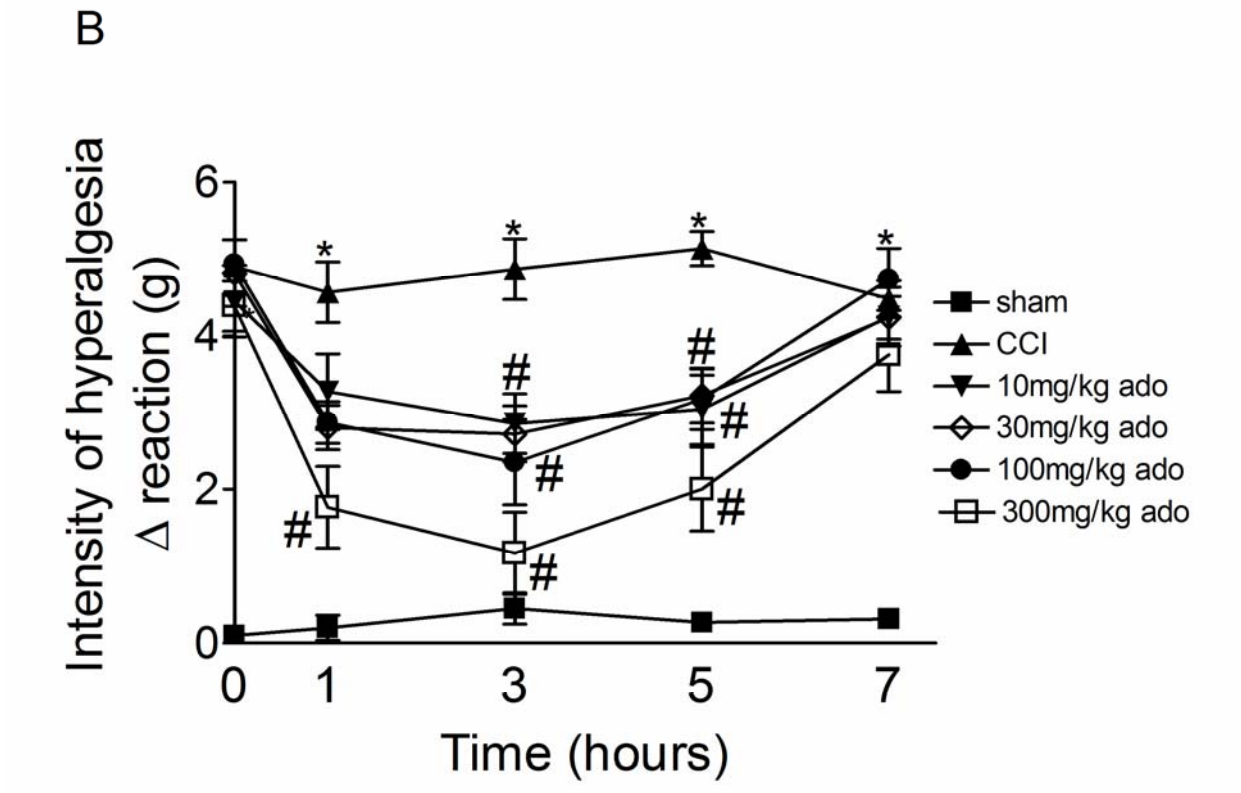
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**Figure 1 -** Fructose, 1-6, biphosphate (FBP) and adenosine (ado) reduced CCI-induced mechanical hyperalgesia in a dose-dependent manner. Seven days after CCI, mice were treated p.o. with FBP (10-300mg/Kg) (A) or ado (10-300mg/kg) (B), and the intensity of hyperalgesia was measured 1-7 h after treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. $^{*}p< 0.05$ compared with the sham group; $^{\#}p< 0.05$ compared with the CCI group and FBP 10 mg/Kg group (A) or compared with the CCI group (B); $^{f}p<0.05$ compared with the CCI, FBP 10 and 30 mg/Kg groups; $^{**}p< 0.05$ compared with the CCI group and FBP 10, 30, 100 mg/Kg groups (one-way ANOVA followed by Bonferroni's t-test).

3.2. Daily treatment with FBP and adenosine reduce CCI-induced mechanical hyperalgesia

Seven days after surgery, it was started a daily treatment with FBP or adenosine (300 mg/kg, p.o.) during 7 days (Fig. 2). The intensity of hyperalgesia was measured before starting the treatment and 3 h after each treatment by the electronic pressure-meter test. Daily treatment with FBP and adenosine significantly reduced CCI-induced mechanical hyperalgesia, demonstrating their applicability for prolonged treatment. Compared with de CCI group, there were significant differences with FBP and adenosine group in all days of treatment, with no significant difference between FBP and adenosine treatments.

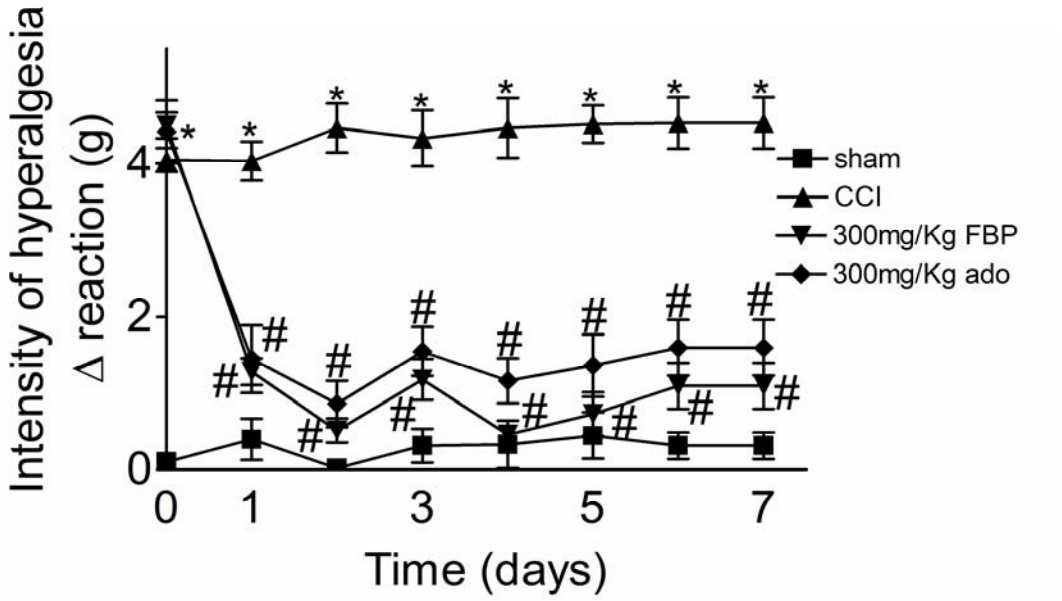


**Figure 2** Daily treatment of FBP and adenosine (ado) reduced CCI-induced mechanical hyperalgesia v.o.. Mice were treated daily (during 7 days) with 300 mg/Kg FBP or ado v.o. after 7 days of CCI surgery. The intensity of hyperalgesia was measured before and 3 h after treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. $^{*}p < 0.05$ compared with the sham group; $^{\#}p < 0.05$ compared with the CCI group (one-way ANOVA followed by Bonferroni's t-test).

3.3. Intrathecal administration of FBP and adenosine - dose-response

Mice were treated i.t. with FBP (3-30 µg/animal) (Fig. 3A) or adenosine (3-30

μg/animal) (Fig. 3B) after 7 days of CCI surgery. The intensity of hyperalgesia was measured before and 1-7 h after treatments by the electronic pressure-meter test to verify the dose required for the anti-hyperalgesic effect of FBP and adenosine in spinal administration.

FBP and adenosine intrathecal administration reduced CCI-induced mechanical hyperalgesia in a dose dependent manner.

In i.t. FBP treatments (Fig. 3A), there was no significant effects with the dose of 3 μg/animal compared with the CCI group in the 1h. In 1h after the treatment, the dose of 10μg/animal was significantly different from the CCI group, the dose of 30 μg/animal was significant different from the CCI and the 3 μg/animal groups. In the 3 h, all FBP doses were significantly different from the CCI group. In the 5 h, the dose of 3 μg/animal was significantly different from the CCI group, the doses of 10 and 30 μg/animal were significantly different from the CCI and 3 μg/animal groups.

In adenosine treatments (Fig. 3B), there were no significant effects with all doses compared with the CCI group in the 1 h. In the 3 h all adenosine groups were different from the CCI group. In the 5 h only the dose of 30 μg/animal were different from the CCI and the 3 μg/animal groups.

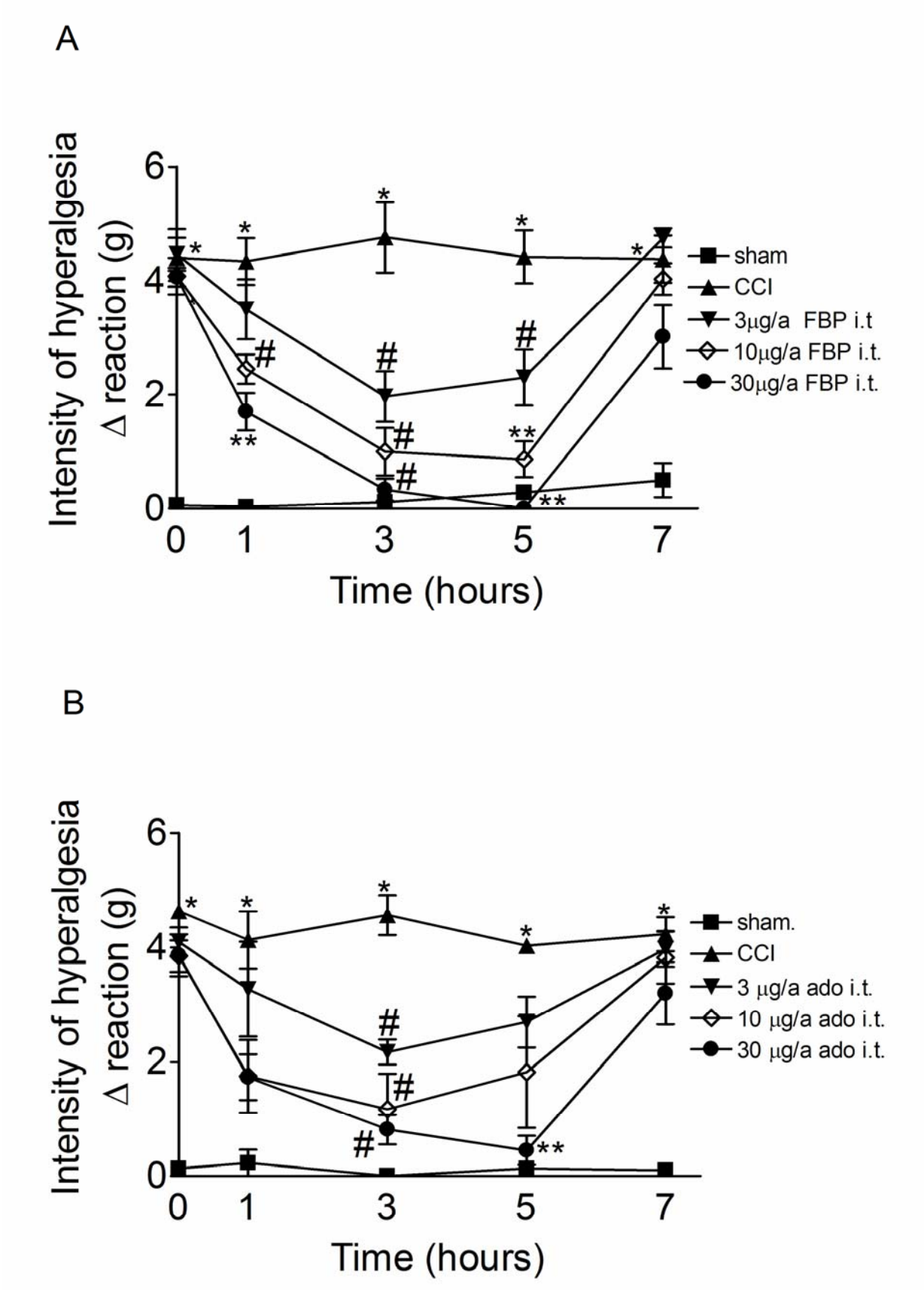
A
Intensity of hyperalgesia
Δ reaction (g)
6
4
2
0
0
1
3
5
7
Time (hours)
sham
CCI
3μg/a FBP i.t
10μg/a FBP i.t.
30μg/a FBP i.t.
B
Intensity of hyperalgesia
Δ reaction (g)
6
4
2
0
0
1
3
5
7
Time (hours)
sham.
CCI
3 μg/a ado i.t.
10 μg/a ado i.t.
30 μg/a ado i.t.

**Figure 3** The i.t. treatment with FBP and adenosine (ado) reduce CCI-induced mechanical hyperalgesia in a dose-dependent manner. Seven days after surgery mice were treated i.t. with FBP (3-30 µg/animal) (A) or ado (3-30 µg/animal) (B). The intensity of hyperalgesia was measured before and 1-7 h after treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. *$p$< 0.05 compared with the sham group; $^{\#}p$< 0.05 compared with the CCI group; **$p$< 0.05 compared with the CCI and the 3 µg/animal FBP (A) or ado (B) groups(one-way ANOVA followed by Bonferroni's t-test).

3.4. Adenosine A1 receptor antagonist reduces ado and FBP anti-hyperalgesic effect

Seven days after CCI surgery mice were treated with DPCPX (A1 receptor antagonist) i.t. (1-10 µg/animal) (Fig. 4A-B) or i.pl. (10 µg/animal) (Fig. 4C-D). Thirty min later mice were treated p.o. with adenosine (300mg/Kg) (Fig. 4A and C) or FBP (300mg/kg) (Fig. 4B and D). The intensity of hyperalgesia was measured before and 1-7 h after treatments by the electronic pressure-meter test. We use the i.t. and i.pl. administration routes of A1 receptor antagonist to verify if adenosine and FBP treatments are dependent on A1 receptor activation in neurons of the spinal cord or peripheral nociceptors.

The doses of 3 and 10µg/animal of DPCPX i.t. abolished adenosine anti-hyperalgesia (Fig. 4A). At 1 h the dose of 1 µg/animal of DPCPX+adenosine was different from the CCI group. At 1h and 5 h the adenosine treatment was significantly different compared to CCI, and 3 and 10 µg/animal of DPCPX+adenosine groups. At 3 h the adenosine group was significantly different compared to CCI and all DPCPX groups. DPCPX presented similar effects in FBP treated groups (Fig. 4B). The doses of 3 and 10µg/animal of DPCPX i.t. completely inhibited FBP effect (Fig. 4B). The dose of 1 µg/animal of DPCPX+FBP was different from the CCI group at 3 h. The FBP treatment was significantly different from the CCI and 10 µg/animal of DPCPX+FBP groups and at 5 h the FBP group was significantly different from CCI and all DPCPX groups. The intraplantar treatment with 10 µg/animal of DPCPX had the same effect of intrathecal administration in adenosine (Fig. 4C) and FBP (Fig. 4D) treated animals, showing that FBP and adenosine present anti-hyperalgesic effect in the CCI model by activating A1 receptors.

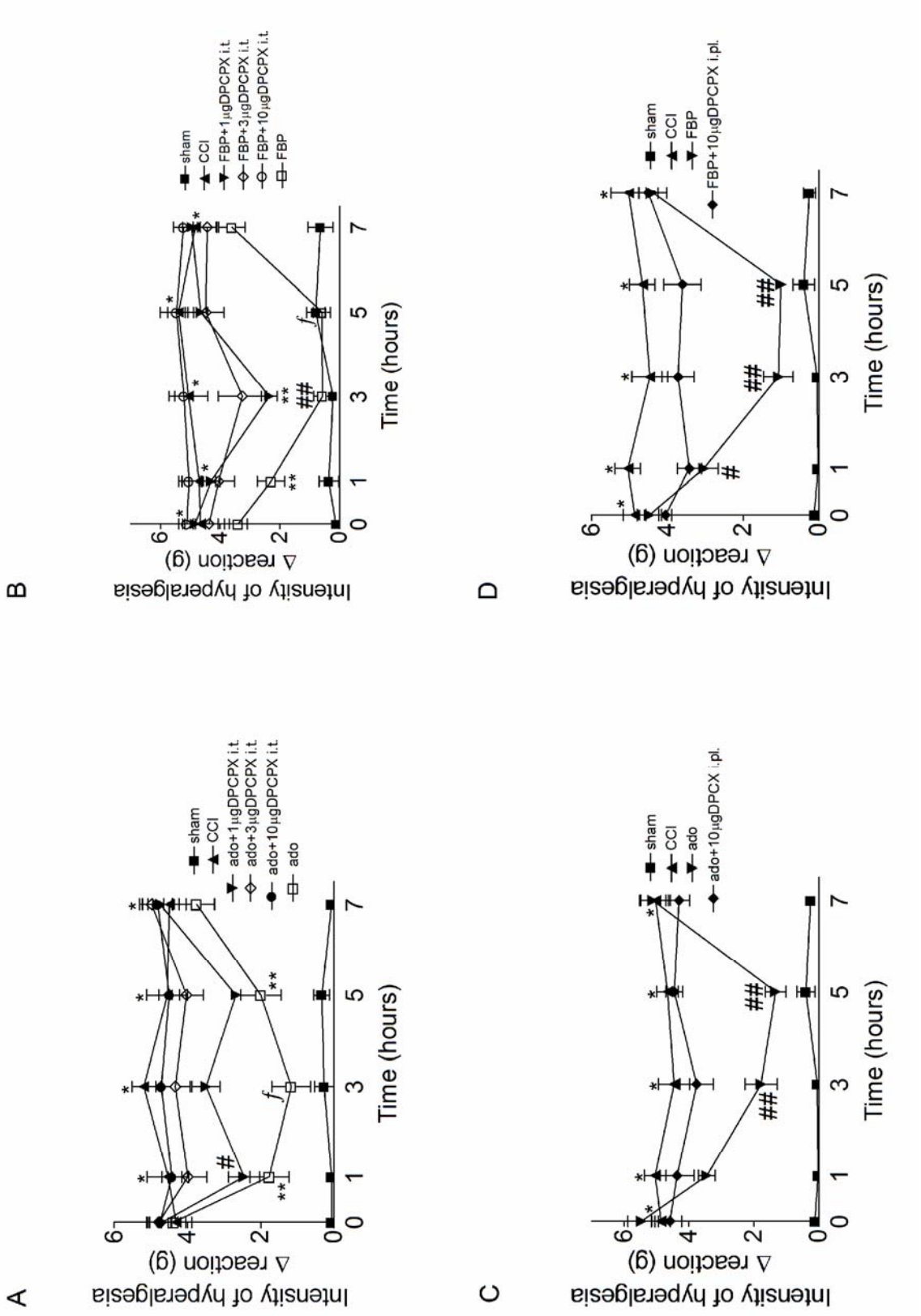


**Figure 4** Adenosine A1 receptor antagonist (DPCPX) reduced the anti-hyperalgesic effect of ado and FBP. Seven days after CCI surgery, mice were treated with DPCPX i.t. (1-10 μg/animal) (A-B) or i.pl. (10 μg/animal) (C-D). Thirty min later mice were

treated p.o. with adenosine (ado) (300mg/Kg) (A and C) or FBP (300mg/kg) (B and D). The intensity of hyperalgesia was measured before and 1-7 h after treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. *$p$< 0.05 compared with the sham group; $^{\#}p$< 0.05 compared with the CCI group; **$p$< 0.05 compared with the CCI, 3, 10 µg/animal DPCPX groups; $^{f}p$< 0.05 compared with CCI, 1, 3, 10 µg/animal DPCPX groups; $^{\#\#}p$< 0.05 compared with the CCI, 10 µg/animal DPCPX groups.

### 3.5. Adenosine A2A receptor antagonist reduces adenosine and FBP anti-hyperalgesic effect

Seven days after CCI surgery, mice were treated with SCH442416 (A2A receptor antagonist) i.t. (0,1-3 µg/animal) (Fig. 5A and 5B) or i.pl. (1 µg/animal) (Fig. 5C and 5D). Thirty minutes later mice were treated p.o. with adenosine (300mg/Kg) (Fig. 5A and 5C) or FBP (300mg/kg) (Fig. 5B and 5D). The intensity of hyperalgesia was measured before and 1-7 h after FBP or adenosine treatments by the electronic pressure-meter test. It was detected that adenosine A2A receptor antagonist reduced the anti-hyperalgesic effect of adenosine and FBP in both routes of administration (Fig. 5). The doses of 1 and 3µg/animal of SCH442416 i.t. abolished ado anti-hyperalgesia (Fig. 5A). At 3 and 5 h the dose of 1 and 3 µg/animal of SCH442416 were different compared to adenosine, and 0.1 and 0.3 µg/animal SCH442416 groups. The group of 0.3 µg/animal of SCH442416 present a small but significant reduction of ado anti-hyperalgesic effect. The dose of 0.1 µg/animal did not present significant antagonism.

In i.t.SCH442416+FBP treatments (Fig. 5B); the doses of 1 and 3µg/animal of SCH442416 i.t. abolished FBP anti-hyperalgesia and were significant compared to the dose of 0.1 µg/animal of SCH442416 (Fig. 5A). These doses were significantly different compared to FBP and 0.1 µg/animal of SCH442416 groups at 3 and 5 h. The dose of 0.3 µg/animal of SCH442416also significantly inhibited FBP anti-hyperalgesia and was significant compared to 0.1 µg/animal of SCH442416 groups at 3 and 5h. The intraplantar treatment of 1 µg/animal SCH442416 had the same effect of intrathecal administration in the adenosine and FBP anti-hyperalgesia.

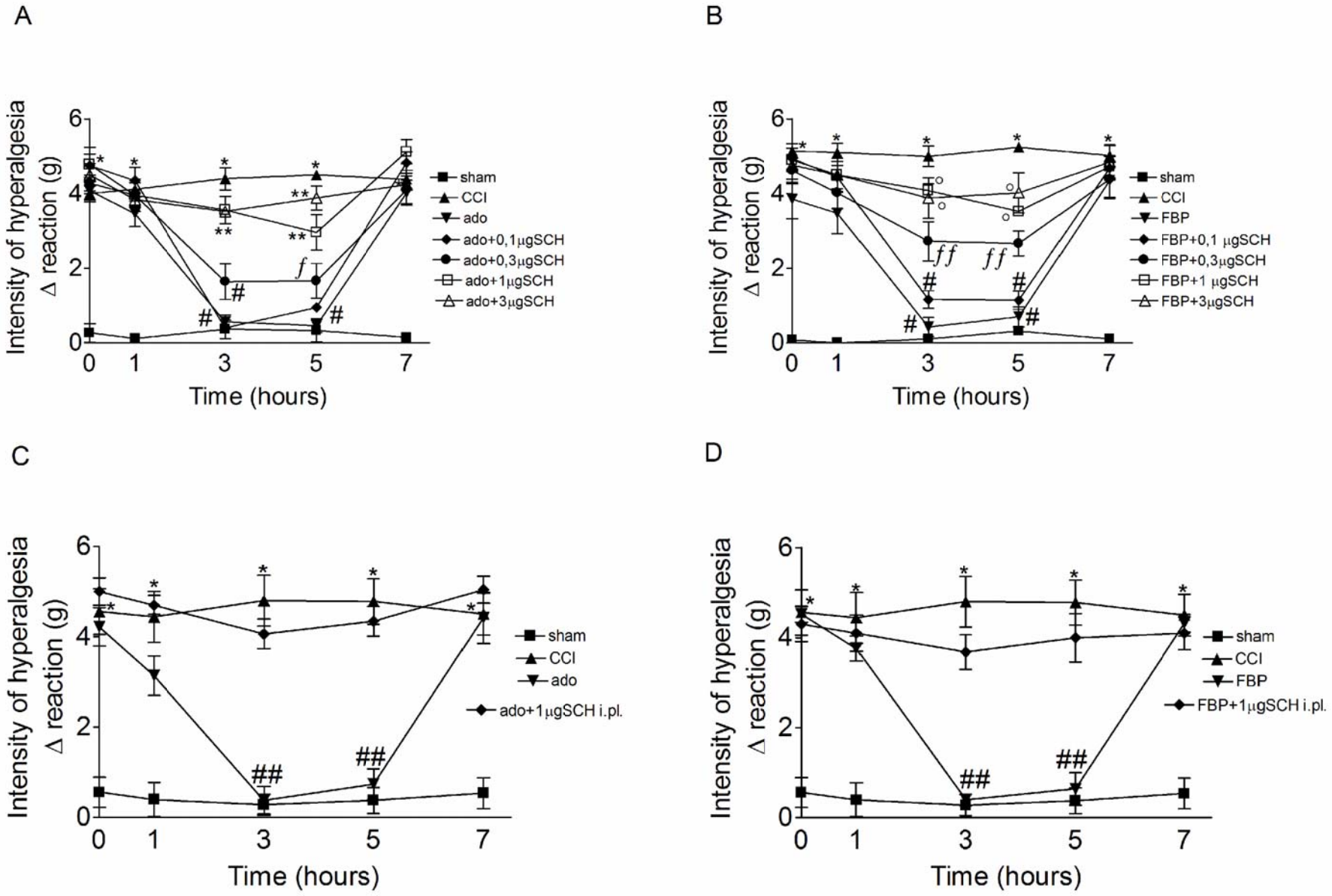
A
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
ado
ado+0,1μgSCH
ado+0,3μgSCH
ado+1μgSCH
ado+3μgSCH
B
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
FBP+0,1 μgSCH
FBP+0,3μgSCH
FBP+1 μgSCH
FBP+3μgSCH
C
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
ado
ado+1μgSCH i.pl.
D
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
FBP+1μgSCH i.pl.

**Figure 5** Adenosine A2 receptor antagonist (SCH442416) reduced the anti-hyperalgesia of adenosine (ado) and FBP in CCI model. Seven days after CCI surgery, mice were treated with SCH442416 i.t. (0.1-3 µg/animal) (A-B) or i.pl. (1 µg/animal) (C-D). Thirty min later mice were treated p.o. with adenosine (300mg/Kg) (A and C) or FBP (300mg/kg) (B and D). The intensity of hyperalgesia was measured before and 1-7 h after FBP or adenosine treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. *$p$< 0.05 compared with the sham group; $^{\#}p$< 0.05 compared with the CCI, 1, 3 µg/animal SCH groups; **$p$< 0.05 compared with the ado, 0.1, 0.3 µg/animal SCH groups; $^{ƒ}p$< 0.05 compared with the CCI, 3 µg/animal SCH groups; $^{\#\#}p$ < 0.05 compared with the CCI, 1 µg/animal SCH groups; $^{ƒƒ}p$< 0.05 compared with the CCI, FBP and 0.1 µg/animal SCH groups; $^{\circ}p$< 0.05 compared with FBP and 0.1 µg/animal SCH groups.

### 3.6. The anti-hyperalgesic effect of FBP in CCI-induced neuropathy depends on activation of NO/cGMP/PKG/K+ATP channels pathway similarly to adenosine

It has been shown that adenosine reduces hyperalgesia by activating the NO/cGMP/PKG/K+ATP channels in neurons (Lima et al., 2010). Considering that FBP administration increases adenosine blood levels to activate adenosine A1 and A2A receptors resulting in reduced hyperalgesia (Valerio et al., 2009; present data), it was verified whether FBP also activates the NO/cGMP/PKG/K+ATP channels pathway. Seven days after surgery, mice were treated with L-NMMA (100 mg/Kg, i.p., 45 min, Fig. 6A), ODQ (1 mg/Kg, i.p., 30 min, Fig. 6B), KT5828 (0,5 µg/animal, i.p., 5 min, Fig. 6C) or glibenclamide (1 mg/Kg, p.o., 30 min, Fig. 6D) before FBP (300 mg/Kg, p.o.) treatment. The intensity of hyperalgesia was measured before and 1-7 h after FBP or ado treatments by the electronic pressure-meter test. The CCI group induced significantly hyperalgesia compared to sham operated group, which was inhibited by FBP as well as the inhibitors of the NO/cGMP/PKG/K+ATP channels pathway reduced the anti-hyperalgesic effect of FBP at 3 and 5 h after FBP treatment.

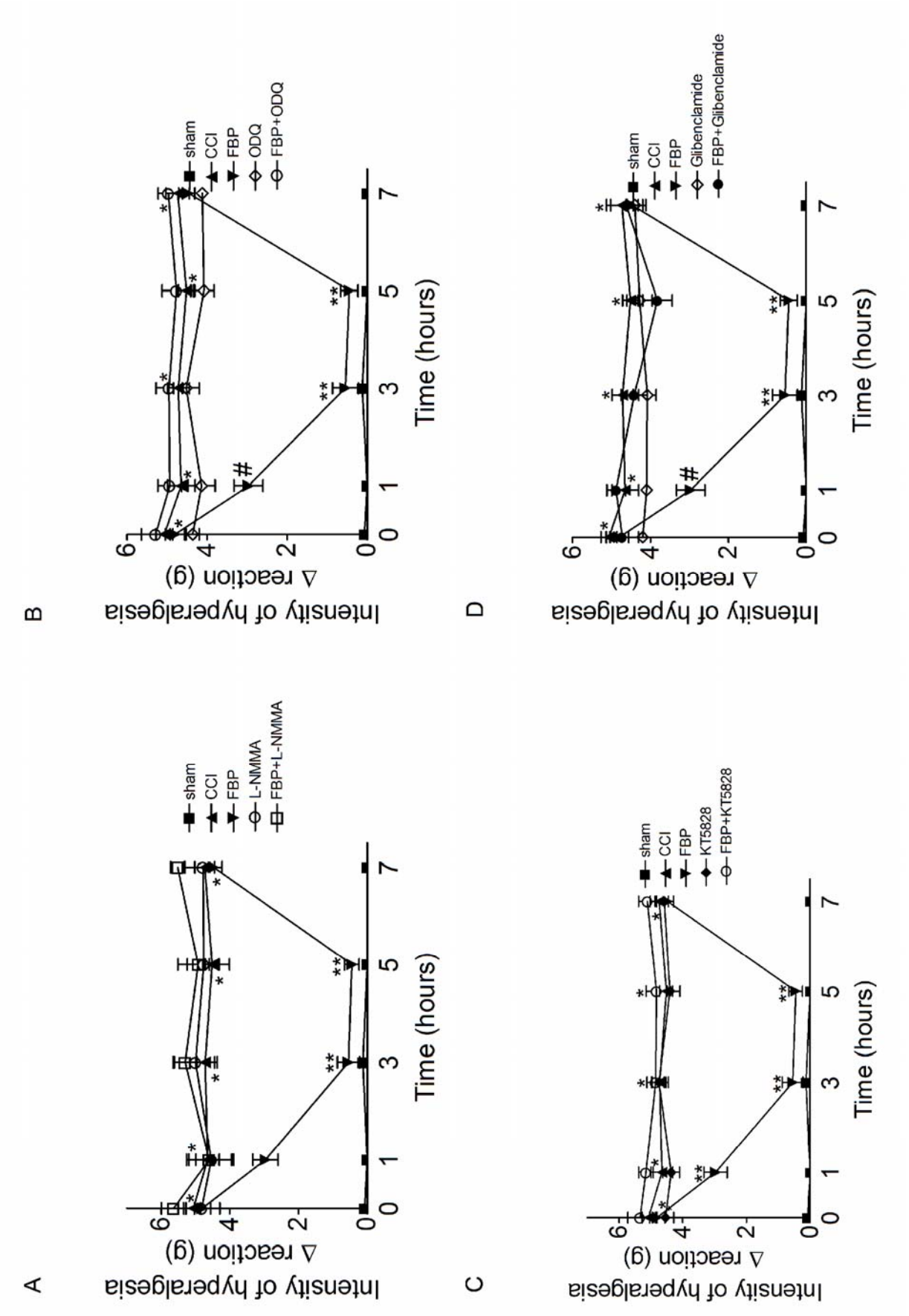
A
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
L-NMMA
FBP+L-NMMA
B
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
ODQ
FBP+ODQ
C
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
KT5828
FBP+KT5828
D
Intensity of hyperalgesia
Δ reaction (g)
Time (hours)
sham
CCI
FBP
Glibenclamide
FBP+Glibenclamide

**Figure 6** The analgesic effect of FBP depends on the activation of the NO/cGMP/PKG/K+ATP channels pathway. Seven days after surgery, mice were treated with L-NMMA (100 mg/Kg, i.p., 45 min, Fig. 6A), ODQ (1 mg/Kg, i.p., 30 min, Fig. 6B), KT5828 (0,5 µg/animal, i.p., 5 min, Fig. 6C) or glibenclamide (1 mg/Kg, p.o., 30 min, Fig. 6D)before FBP (300 mg/Kg, p.o.) treatment. The intensity of hyperalgesia was measured before and 1-7 h after FBP or ado treatments by the electronic pressure-meter test. n = 6 mice per group per experiment. *$p$< 0.05 compared with the sham group; $^{\#}p$< 0.05 compared with CCI and FBP+ODQ groups (B) or CCI and FBP+glibenclamide groups (D); **$p$< 0.05 compared with CCI, L-NMMA and FBP+L-NMMA groups (A) or CCI, ODQ and FBP+ODQ groups (B) or ICC, KT5828 and FBP+KT5828 groups (C) or CCI, glibenclamide and FBP+glibenclamide groups (D) (one-way ANOVA followed by Bonferroni's t-test).

## 4. Discussion

We have previously demonstrated that FBP reduces carrageenin-induced mechanical hyperalgesia by increasing the levels of adenosine, which in turn, activates adenosine A1 receptors (Valério et al., 2009). In turn, the activation of A1 receptors triggers the NO/cGMP/PKG/K+ATP channels signaling pathway (Lima et al. 2010). Furthermore, adenosine reduces neuropathic pain by activating A1 and A2A receptors. Thus, we reason that FBP could diminish neuropathic pain by increasing adenosine levels, therefore, activating adenosine receptors. In fact, FBP reduced CCI-induced neuropathic pain in a similar fashion as adenosine, which was prevented by adenosine A1 and A2A receptor antagonists and inhibitors of the NO/cGMP/PKG/K+ATP channels signaling pathway.

The relation between FBP and adenosine was first investigated by Sola et al., 2003, they showed that the anti-inflammatory actions of FBP in a model of ischaemia/reperfusion of intestine were reversed by treatment with adenosine deaminase, which is an enzyme that converts adenosine to an inative metabolite. Valério et al., 2009 provided more evidence that adenosine is crucial for the pharmacological effect of FBP as they showed that the treatment with A1 receptor antagonist, prevented the anti-hyperalgesic effect of FBP and that the administration of FBP increased the levels of adenosine in blood by HPLC. The following mechanism was suggested: as FBP causes an increase in the intermediates of

glycolysis, which induces an efflux of AMP from the cell, this AMP can be hydrolysed to adenosine in the extracellular environment, which would then be available to bind to adenosine receptors.

High concentrations of adenosine receptor agonists can have unacceptable side effects in vivo, inducing hemodynamic responses such hypotension, bradycardia, and atrioventricular block. (Barankiewicz et al., 1997; Monopoli et al., 1996). So alternatives have been tested to raise adenosine concentrations endogenously (Barankiewicz et al., 1997).

FBP seems to be a good alternative, not just because it is a drug that can increase endogenously adenosine, which is already used in this treatment, but because of the well-documented pharmacological effects of FBP that can be linked to the physiopathology of neuropathic pain; obviously some of these FBP pharmacological effects may be because of the adenosine production, but this must be more investigated.

Adenosine can bind four different receptors: adenosine A1, A2A, A2B, and A3. A1 and A3 receptors are coupled to pertussis toxin-inhibited Gi/o proteins, the activation of which results decreases intracellular cAMP levels. A2A and A2B receptors couple to Gs proteins and stimulate adenylyl cyclase and cAMP accumulation (Fredholm et al., 2000 and 2001). This receptor complexity reflects the multifaceted role that adenosine has in health and disease depending on receptor distribution in different cells in the body.

Some studies have explored the effect of A1 receptors agonists, as A1 receptors are found predominantly on neurons (Hasko et al., 2007) and A1 receptors agonists are antinociceptive in a number of different pain models (Lee and Yaksh, 1996; Yamamoto et al., 2003; Zahn et al., 2007) including neuropathic pain (Cui et al., 1997; Balasubramanyan et al., 2008; Curros-Criado et al., 2005; Gong et al., 2010).

There is evidence of inhibitory actions mediated by adenosine A1 receptors on primary afferent nerve terminals, on interneurons and on projection neurons in the dorsal spinal cord (Santicioli et al., 1993; Lao et al., 2001; Patel et al., 2001).

In the present experiments we showed that the inhibition of A1 receptor with DPCPX in both routes – intrathecal and intraplantar lead to the inhibition of FBP and adenosine effect. Suggesting that adenosine acts on A1 receptor in peripheral and central neurons.

A2A receptor agonists may be of special interest. A growing body of evidence is

presenting A2A receptor agonists as having potent anti-inflammatory and immunoregulatory effects on peripheral immune cells, including suppression of proinflammatory cytokines and enhanced production of the anti-inflammatory cytokine, IL-10 (Thiel et al., 2003; Hasko and Cronstein, 2004; Sitkovsky et al., 2004). Loram et al., 2009 showed that intrathecal injection of A2A receptor agonists reversed neuropathic pain and this effect likely involved suppression of astrocyte, which are cells with immunogenic properties, and microglial activation, which are the immuno-competent macrophages of the CNS (Ren and Dubner, 2008). Loram et al. reported that the attenuation of neuropathic pain was mediated by A2A receptor activation initially, but the sustained reversal of allodynia is likely mediated by IL-10 release, possibly from resident or recruited cells in the intrathecal space.

A2A receptors activation leads to generation of intracellular cAMP, and activation of protein kinase A (PKA), which can then phosphorylate and thereby activate the transcription factor CREB (Nemeth et al., 2003), directly affecting gene expression by binding to gene promoters or indirectly, by competing with NF-kB for an important cofactor, CREB-binding protein (Fredholm et al., 2007). In addition, it was demonstrated that another transcription factor, CCAAT enhancer-binding protein beta, is responsible for the stimulatory effect of A2A receptor agonists on IL-10 production (Csoka et al., 2007)

On the other hand, there is evidence that A2A receptor activation can be pro-nociceptive especially when it is administrated peripherally (Taiwo and Levine, 1990; Doak and Sawynok, 1995; Khasar et al., 1995).

In the present results, the intrathecal and intraplantar administration of A2A receptor antagonist inhibited the anti-hyperalgesic effect of adenosine and FBP. Although our result seems to be contradicting the literature, there is evidence that systemic administration of A2A receptors antagonist reduces the antinociceptive effect of inosine (a metabolite of adenosine) (Nascimento et al., 2010) and the analgesic effect of adenosine in the formalin test (Doak and Sawynok, 1995). Moreover, A2A receptor induced analgesia in formalina test (Borghi et al., 2002), which are in agreement with the present data.

Recently, it was demonstrated that adenosine reduces carrageenin-induced mechanical hyperalgesia in adenosine A1 receptor dependent activation of NO/cGMP/PKG/K+ATP channels signaling pathway (Lima et al., 2010).

This sinaling payway of NO was evidencied in early 1990s and is the mechanism of action of several analgesic compounds (Cury et al., 2011).

Similar effects were observed using plantar and spinal administration of adenosine A1 and A2A receptor antagonists to inhibit FBP and adenosine anti-hyperalgesia. This observation resembles the teleantagonism phenomenum, for instance, the i.t. or i.pl. administration of IL-1β induces hyperalgesia, which was inhibited by treatment with indomethacin (cyclooxygenase inhibitor) in the opposite site (Funez et al., 2008). Demonstrating that for some mediators, the stimulation in the spinal cord can be inhibited in a peripheral site (paw) as well as peripheral stimulation can be inhibited by intrathecal treatment.

In addition to the regulation of neuronal activity FBP would affect other related molecules and cells that were not investigated in the present study. Calafell et al., 2009, examined the intracellular mechanisms involved in the FBP inhibition of the apoptosis induced by TNF-α in parenchyma cells of galactosamine-sensitized rat liver, a model of experimental hepatitis. They showed a reduction in apoptosis concomitant with an increase in nitric oxide (NO) production. In such conditions, guanylyl cyclase is activated and the increase in cGMP reduces the TNFα-induced apoptosis in hepatocytes.

If FBP inhibits TNF-α production, this could be an important mechanism of FBP since TNF-α increases TTX-R Na+ channels currents in nociceptive dorsal root ganglia neurons (Jin X and Gereau, 2006) and increase voltage-activated sodium channels currents (Czeschik et al., 2008) leading to overall neuronal hyper-excitability and hence leading to neuropathic pain.

Furthermore, Yin H et al., 2008, reported that a in vitro pretreatment with FBP remarkably repressed the production of TNF-α in murine alveolar macrophages exposed to LPS in a model of acute lung injury, suggesting that FBP suppressed the nuclear translocation of NFκB in lung tissues in response to LPS challenge.

Some authors associated the FBP treatment with the increase of NO production (Rao et al., 1998; Mihas et al., 1997 and 2003) corroborating our finding that L-NMMA reduced the anti-hyperalgesic effect of FBP. The beneficial effect of NO overproduction requires concomitant inhibition of free-radical production in the cells (Meurer et al., 2005; Gerassimou et al., 2007). In addition, an excess of NO in an oxidative milieu generates the hyper-reactive free-radical peroxynitrite, which increases oxidative stress and activates apoptosis pathways (Guzik et al., 2002).

Calafell et al., 2009 showed that FBP reduced free-radical production and increased the GSH/GSSG ratio. Others authors also demonstrated in many models the capacity of FBP to reduce oxidative stress (Vexler et al., 2003; Ahn et al., 2007; Gámez et al., 2008; Xu et al., 2010).

Under conditions of low oxidative stress, such as FBP does, soluble guanylyl cyclase is the major target of the overproduced NO in the cell (Meurer et al., 2005; Gerassimou et al., 2007) which converts GTP into the second messenger cGMP (Cary et al., 2006). cGMP inhibits NADPH oxidase activity, mitochondrial permeability transition and cytochrome c release, through activation of cGMP-dependent protein kinase pathway, and thus reduced TNFα-induced apoptosis (Li et al., 2000; Kim et al., 2004; Wang et al., 2006).

In conclusion, the present study demonstrates that: (i) FBP and adenosine inhibited the mechanical hyperalgesia induced by CCI with a similar profile; (ii) the antinociceptive effect of FBP and adenosine are dependent on activation of A1 and A2A receptors; (iii) FBP treatment promote antinociception through NO/cGMP/PKG/KATP signaling pathway similarly to adenosine (Lima et al., 2010). Therefore, these results further support the importance of further investigations on the potential analgesic effect of FBP in neuropathic pain.

**References**

Ahn, S.M., Hwang, J.S., Lee, S.H., 2007. Fructose 1,6-diphosphate alleviates UV-induced oxidative skin damage in hairless mice. Biol. Pharm. Bull. 30(4), 692-7.

Azambuja, A.A., Lunardelli, A., Nunes, F.B., Gaspareto, P.B., Donadio, M.V., de Figueiredo, C.E., de Oliveira, J.R., 2011. Effect of fructose-1,6-bisphosphate on the nephrotoxicity induced by cisplatin in rats. Inflammation 34(1), 67-71.

Balasubramanyan, S., Sharma, S.S., 2008. Protective effect of adenosine in diabetic neuropathic pain is mediated through adenosine A1-receptors. Indian J. Physiol. Pharmacol. 52(3), 233-42.

Barankiewicz, J., Danks, A.M., Abushanab, E., Makings, L., Wiemann, T., Wallis, R.A., Pragnacharyulu, P.V., Fox, A., Marangos, P.J., 1997. Regulation of adenosine concentration and cytoprotective effects of novel reversible adenosine deaminase inhibitors. J. Pharmacol. Exp. Ther. 283, 1230 –1238.

Belfrage, M., Segerdahl, M., Arnér, S., Sollevi, A., 1999. The safety and efficacy of intrathecal adenosine in patients with chronic neuropathic pain. Anesth. Analg. 89(1), 136-142.

Belfrage, M., Sollevi, A., Segerdahl, M., Sjölund, K.F., Hansson, P., 1995. Systemic adenosine infusion alleviates spontaneous and stimulus evoked pain in patients with peripheral neuropathic pain. Anesth. Analg. 81(4),713-117.

Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33(1), 87-107.

Borghi, V., Przewlocka, B., Labuz, D., Maj, M., Ilona, O., Pavone, F., 2002. Formalin-induced pain and mu-opioid receptor density in brain and spinal cord are modulated by A1 and A2a adenosine agonists in mice. Brain Res. 956(2), 339-48.

Calafell, R., Boada, J., Santidrian, A.F., Gil, J., Roig, T., Perales, J.C., Bermudez, J., 2009. Fructose 1,6-bisphosphate reduced TNF-alpha-induced apoptosis in galactosamine sensitized rat hepatocytes through activation of nitric oxide and cGMP production. Eur. J. Pharmacol. 610(1-3), 128-33.

Cary, S.P., Winger, J.A., Derbyshire, E.R., Marletta, M.A., 2006. Nitric oxide signaling: no longer simply on or off. Trends Biochem. Sci. 31, 231–239.

Csoka, B., Nemeth, Z.H., Virag, L., Gergely, P., Leibovich, S.J., Pacher, P., Sun, C. X., Blackburn, M.R., Vizi, E.S., Deitch, E.A., Hasko, G., 2007. A2A adenosine receptors and C/EBP beta are crucially required for IL-10 production by macrophages exposed to E. coli. Blood 110, 2685– 2695.

Cui, J.G., Sollevi, A., Linderoth, B., Meyerson, B.A., 1997. Adenosine receptor activation suppresses tactile hypersensitivity and potentiates spinal cord stimulation in mononeuropathic rats. Neurosci. Lett. 223(3), 173-6.

Cunha, T.M., Verri, W.A.Jr., Vivancos, G.G., Moreira, I.F., Reis, S., Parada, C.A., Cunha, F.Q., Ferreira, S.H. 2004. An electronic pressure-meter nociception paw test for mice. Braz. J. Med. Biol. Res. 37(3), 401-7.

Curros-Criado, M.M., Herrero, J.F., 2005. The antinociceptive effects of the systemic adenosine A1 receptor agonist CPA in the absence and in the presence of spinal cord sensitization. Pharmacol. Biochem. Behav. 82(4), 721-6.

Cury, Y., Picolo, G., Gutierrez, V.P., Ferreira, S.R., 2011. Pain and analgesia: The dual effect of nitric oxide in nociceptive system. Nitric Oxide 25, 243-54.

Czeschik, J.C., Hagenacker, T., Schafers, M., Busselberg, D., 2008. TNF-alpha differentially modulates ion channels of nociceptive neurons. Neurosci. Lett. 434, 293-298.

de Oliveira, L.M., Pires, M.G., Magrisso, A.B., Munhoz, T.P., Roesler, R., de Oliveira, J.R., 2010. Fructose-1,6-bisphosphate inhibits in vitro and ex vivo platelet aggregation induced by ADP and ameliorates coagulation alterations in experimental sepsis in rats. J. Thromb. Thrombolysis 29(4), 387-94.

Ding, Y., Wang, S., Zhang, M.M., Guo, Y., Yang, Y., Weng, S.Q., Wu, J.M., Qiu, X., Ding, M.P., 2010. Fructose-1,6-diphosphate inhibits seizure acquisition in fast hippocampal kindling. Neurosci. Lett. 477(1), 33-6.

Doak, G.J., Sawynok, J., 1995. Complex role of peripheral adenosine in the genesis of the response to subcutaneous formalin in the rat. Eur. J. Pharmacol. 281:311–318.

Eisenach, J.C., Curry, R., Hood, D.D., 2002. Dose response of intrathecal adenosine in experimental pain and allodynia. Anesthesiol. 97(4), 938-942.

Eisenach, J.C., Rauck, R.L., Curry, R., 2003. Intrathecal, but not intravenous adenosine reduces allodynia in patients with neuropathic pain. Pain 105(1-2), 65-70.

Fredholm, B.B., Arslan, G., Halldner, L., Kull, B., Schulte, G., Wasserman, W., 2000. Structure and function of adenosine receptors and their genes. Naunyn. Schmiedebergs. Arch. Pharmacol. 362, 364–374.

Fredholm, B.B., Chern, Y., Franco, R., Sitkovsky, M., 2007. Aspects of the general biology of adenosine A2A signaling. Prog. Neurobiol. 83, 263–276.

Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

Funez, M.I., Ferrari, L.F., Duarte, D.B., Sachs, D., Cunha, F.Q., Lorenzetti, B.B., Parada, C.A., Ferreira, S.H., 2008. Teleantagonism: A pharmacodynamic property of the primary nociceptive neuron. Proc. Natl. Acad. Sci. U.S.A. 105(49), 19038-43.

Gámez, A., Alva, N., Roig, T., Bermúdez, J., Carbonell, T., 2008. Beneficial effects of fructose-1,6-biphosphate on hypothermia-induced reactive oxygen species injury in rats. Eur. J. Pharmacol. 590(1-3), 115-9.

Gawarammana, I., Mohamed, F., Bowe, S.J., Rathnathilake, A., Narangoda, S.K., Azher, S., Dawson, A.H., Buckley, N.A., 2010. Fructose-1, 6-diphosphate (FDP) as a novel antidote for yellow oleander-induced cardiac toxicity: a randomized controlled double blind study.BMC Emerg. Med. 29,10-15.

Gerassimou, C., Kotanidou, A., Zhou, Z., Simoes, D.D.C., Roussos, C., Papapetropoulos, A., 2007. Regulation of the expression of soluble guanylylcyclase by reactive oxygen species. Br. J. Pharmacol. 150, 1084–1091.

Gong, Q.J., Li, Y.Y., Xin, W.J., Wei, X.H., Cui, Y., Wang, J., Liu, Y., Liu, C.C., Li, Y.Y., Liu, X.G., 2010. Differential effects of adenosine A1 receptor on pain-related behavior in normal and nerve-injured rats. Brain Res. 1361, 23-30.

Guzik, T.J., West, N.E.J., Pillai, R., Taggart, D.P., Channon, K.M., 2002. Nitric oxide modulates superoxide release and peroxynitrite formation in human blood vessels. Hypertension 39, 1088–1094.

Hasko, G., Cronstein, B.N., 2004. Adenosine: an endogenous regulator of innate immunity. Trends Immunol. 25, 33–39

Hasko, G., Pacher, P., Deitch, E.A., Vizi, E.S., 2007. Shaping of monocyte and macrophage function by adenosine receptors. Pharmacol. Ther. 113, 264 –275.

Inoue, K., Tsuda, M., Tozaki-Saitoh H., 2007. Modification of neuropathic pain sensation through microglial ATP receptors. Purinergic. Signal.3(4), 311-6.

Jin, X., Gereau, R.W., 2006. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J. Neurosci 26, 246-255.

Kaakinen, T., Heikkinen, J., Dahlbacka, S., Alaoja, H., Laurila, P., Kiviluoma, K., Salomäki, T., Romsi, P., Tuominen, H., Biancari, F., Lepola, P., Nuutinen, M., Juvonen, T., 2006. Fructose-1,6-bisphosphate supports cerebral energy metabolism in pigs after ischemic brain injury caused by experimental particle embolization. Heart Surg. Forum 9(6), E828-35.

Khasar, S.G., Wang, J.F., Taiwo, Y.O., Heller, P.H., Green, P.G., Levine, J.D., 1995. Mu-opioid agonist enhancement of prostaglandin-induced hyperalgesia in the rat: a G-protein beta gamma subunit-mediated effect? Neurosc. 67, 189–195.

Kim, J.S., Ohshima, S., Pediaditakis, P., Lemasters, J.J., 2004. Nitric oxide protects rat hepatocytes against reperfusion injury mediated by the mitochondrial permeability transition. Hepatol. 39, 1533–1543.

Lao, L.J., Kumamoto, E., Luo, C., Furue, H., Yoshimura, M., 2001. Adenosine inhibits excitatory transmission to substantia gelatinosa neurons of the adult rat spinal cord through the activation of presynaptic A(1) adenosine receptor.Pain 94(3), 315-24.

Lee, Y.W., Yaksh, T.L., 1996. Pharmacology of the spinal adenosine receptor which mediates the antiallodynic action of intrathecal adenosine agonists. J. Pharmacol. Exp. Ther. 277, 1642–1648.

Lian, X.Y., Xu, K., Stringer, J.L., 2008. Oral administration of fructose-1,6-diphosphate has anticonvulsant activity. Neurosci. Lett. 446(2-3), 75-7.

Li, J., Yang, S., Billiar, T.R., 2000. Cyclic nucleotides suppress tumor necrosis factor alpha -mediated apoptosis by inhibiting caspase activation and cytochrome c release in primary hepatocytes via a mechanism independent of Akt activation. J. Biol. Chem. 275, 13026–13034.

Lima, F.O., Souza, G.R., Verri, W.A. Jr., Parada, C.A., Ferreira, S.H., Cunha, F.Q., Cunha, T.M., 2010. Direct blockade of inflammatory hypernociception by peripheral A1 adenosine receptors: involvement of the NO/cGMP/PKG/KATP signaling pathway. Pain 151(2), 506-15.

Loram, L.C., Harrison, J.A., Sloane, E.M., Hutchinson, M.R., Sholar, P., Taylor, F.R., Berkelhammer, D., Coats, B.D., Poole, S., Milligan, E.D., Maier, S.F., Rieger, J., Watkins, L.R., 2009. Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine2Areceptor agonists: a novel therapy for neuropathic pain. J. Neurosci. 29(44),14015-25.

Lynch, M.E., Clark, A.J., Sawynok, J., 2003. Intravenous adenosine alleviates neuropathic pain: a double blind placebo controlled crossover trial using an enriched enrolment design. Pain 103(1-2), 111-117.

Meurer, S., Pioch, S., Gross, S., Muller-Esterl, W., 2005. Reactive oxygen species induce tyrosine phosphorylation of and Src kinase recruitment to NO-sensitive guanylylcyclase. J. Biol. Chem. 280, 33149–33156.

Mihas, A.A., Kanji, V.K., Mihas, T.A., Joseph, R.M., Markov, A.K., Heuman, D.M., 2003. Fructose diphosphate attenuates the acetaminophen-induced liver injury in the rat evidence for involvement of nitric oxide. Res. Commun. Mol. Pathol. Pharmacol. 114, 253-66.

Mihas, A.A.,Maliakkal, R.J., Mihas, T.A., Kanji, V.K., Markov, A.K., 1997. Effects of fructose-1,6-diphosphate on the activity of rat liver nitric oxide synthase in vitro. Pharmacol. 54(1), 43-8.

Monopoli, A., Conti, A., Zocchi, C., 1996. Pharmacology of the new selective A2A adenosine receptor agonist 2-hexynyl-5′-N-ethylcarboxamido adenosine. Arzneimittel-Forschung 44, 1296–304.

Nascimento, F.P., Figueredo, S.M., Marcon, R., Martins, D.F., Macedo, S.J.Jr., Lima, D.A., Almeida, R.C., Ostroski, R.M., Rodrigues, A.L., Santos, A.R., 2010. Inosine reduces pain-related behavior in mice: involvement of adenosine A1 and A2A receptor subtypes and protein kinase C pathways. J. Pharmacol. Exp. Ther.334(2), 590-8.

Nemeth, Z.H., Leibovich, S.J., Deitch, E.A., Sperlagh, B., Virag, L., Vizi, E.S., Szabo, C., Hasko, G., 2003. Adenosine stimulates CREB activation in macrophages via a p38 MAPK-mediated mechanism. Biochem. Biophys. Res. Commun. 312, 883–888.

Patel, M.K., Pinnock, R.D., Lee, K., 2001. Adenosine exerts multiple effects in dorsal horn neurones of the adult rat spinal cord. Brain Res. 920(1-2), 19-26.

Rao, M.R., Olinde, K.D., Markov, A.K., 1998. In vitro induction of nitric oxide by fructose-1,6-diphosphate in the cardiovascular system of rats. Mol. Cell. Biochem. 185(1-2), 171-5.

Regaya, I., Pham, T., Andreotti, N., Sauze, N., Carregam, L., Martin-Eauclaire, M.F., Jouirou, B., Peragut, J.C., Vacher, H., Rochat, H., Devaux, C., Sabatier, J. M., Guieu, R., 2004. Small conductance calcium-activated K+ channels, SkCa, but not voltage-gated K+ (Kv) channels, are implicated in the antinociception induced by CGS21680, a A2A adenosine receptor agonist. Life Sci. 76(4), 367-377.

Ren, K., Dubner, R., 2008. Neuron-glia crosstalk gets serious: role in pain hypersensitivity. Curr. Opin. Anaesthesiol. 21, 570 –579.

Riedel, B.J., Gal, J., Ellis, G., Marangos, P. J., Fox, A.W., Royston, D. 2004. Myocardial protection using fructose-1,6-diphosphate during coronary artery bypass graft surgery: a randomized, placebo-controlled clinical trial. Anesth. Analg. 98(1), 20-9.

Santicioli, P., Del Bianco, E., Maggi, C.A., 1993. Adenosine A1 receptors mediate the presynaptic inhibition of calcitonin gene-related peptide release by adenosine in the rat spinal cord. Eur. J. Pharmacol. 231(1), 139-42.

Scholz, J., Woolf, C.J., 2007. The neuropathic pain triad: Neurons, immune cells and glia. Nat. Neurosci. 10, 1361–1368.

Sitkovsky, M.V., Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., Ohta, A., Thiel, M., 2004. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. 22, 657–682.

Sjölund, K.F., Belfrage, M., Karlsten, R., Segerdahl, M., Arnér, S., Gordh, T., Solevi, A., 2001. Systemic adenosine infusion reduces the area of tactile allodynia in neuropathic pain following peripheral nerve injury: a multi-centre, placebo-controlled study. Eur. J. Pain 5(2), 199-207.

Sola, A., Panés, J., Xaus, C., Hotter, G,. 2003. Fructose-1,6-biphosphate and nucleoside pool modifications prevent neutrophil accumulation in the reperfused intestine. J. Leukoc. Biol. 73(1), 74-81.

Song, X., Wu, B., Takata, T., Wang, X., Oizumi, X.S., Akisaki, T., Yokono, K., Sakurai, T., 2005. Neuroprotective effect of D-fructose-1,6-bisphosphate against beta-amyloid induced neurotoxicity in rat hippocampal organotypic slice culture: involvement of PLC and MEK/ERK signaling pathways. Kobe J. Med. Sci. 51(5-6), 73-83.

Thiel, M., Caldwell, C.C., Sitkovsky, M.V., 2003. The critical role of adenosine A2A receptors in downregulation of inflammation and immunity in the pathogenesis of infectious diseases. Microbes Infect. 5, 515–526.

Valério, D.A., Ferreira, F.I., Cunha, T.M., Alves-Filho, J.C., Lima, F.O., De Oliveira, J.R., Ferreira, S.H., Cunha, F.Q., Queiroz, R.H., Verri, W.A.Jr., 2009. Fructose-1,6-bisphosphate reduces inflammatory pain-like behaviour in mice: role of adenosine acting on A1 receptors. Br. J. Pharmacol. 158(2), 558-68.

Vale, M.L., Rolim, D.E., Cavalcante, I.F., Ribeiro, R.A., Souza, M.H., 2007. Role of NO/cGMP/KATP pathway in antinociceptive effect of sildenafil in zymosan writhing response in mice. Inflamm. Res. 56(2), 83-8.

Vexler, Z.S., Wong, A., Francisco, C., Manabat, C., Christen, S., Täuber, M., Ferriero, D.M., Gregory, G., 2003. Fructose-1,6-bisphosphate preserves intracellular glutathione and protects cortical neurons against oxidative stress. Brain Res. 960(1-2), 90-8.

Wang, Y., Kim, P.K., Peng, X., Loughran, P., Vodovotz, Y., Zhang, B., Billiar, T.R., 2006. Cyclic AMP and cyclic GMP suppress TNFalpha-induced hepatocyte apoptosis by inhibiting FADD up-regulation via a protein kinase A-dependent pathway. Apoptosis 11, 441–451.

Watkins, L.R., Hutchinson, M.R., Ledeboer, A., Wieseler-Frank, J., Milligan, E.D., Maier, S.F., 2007. Norman Cousins Lecture. Glia as the “bad guys”: implications for improving clinical pain control and the clinical utility of opioids. Brain Behav. Immun. 21, 131–146.

Xu, M., Dai, D.Z., Zhang, Q., Cheng, Y.S., Dai, Y., 2010. Upregulated NADPH oxidase contributes to diabetic testicular complication and is relieved by strontium fructose 1,6-diphosphate. Exp. Clin. Endocrinol. Diabetes 118(7), 459-65.

Yamamoto, S., Nakanishi, O., Matsui, T., Shinohara, N., Kinoshita, H., Lambert, C., Ishikawa, T., 2003. Intrathecal adenosine A1 receptor agonist attenuates hyperalgesia without inhibiting spinal glutamate release in the rat. Cell. Mol. Neurobiol. 23,175–185.

Yin, H., Jin, X.B., Gong, Q., Yang, H., Hu, L.Y., Gong, F.L., Zhu, J.Y., 2008. Fructose-1,6-diphosphate attenuates acute lung injury induced by lipopolysaccharide in mice.Int. Immunopharmacol. 8(13-14):1842-7.

Zahn, P.K., Straub, H., Wenk, M., Pogatzki-Zahn, E.M., 2007. Adenosine A1 but not A2a receptor agonist reduces hyperalgesia caused by a surgical incision in rats: a pertussis toxin-sensitive G protein-dependent process. Anesthesiol. 107, 797– 806.

Zhang, Q., Liu, H.R., Ying, H.J., Dai, D.Z., Tang, X.Y., Dai, Y., 2009. Strontium fructose-1,6-diphosphate alleviates early diabetic testopathy by suppressing abnormal testicular matrix metalloproteinase system in streptozocin-treated rats. J. Pharm. Pharmacol. 61(2), 229-36.

# ANEXO

## ANEXO A

Highlights

Highlights are mandatory for Neuropharmacology Journal.
It include 3 to 5 bullet points with maximum 85 characters, including spaces, per bullet point.

**HIGHLIGHTS**

- FBP and adenosine inhibited hyperalgesia induced by CCI with a similar profile; [82 characters]
- Antinociceptive effect of FBP is dependent on activation of A1 and A2A receptors; [84 characters]
- FBP treatment promotes antinociception through NO/cGMP/PKG/KATP signaling pathway; [85 characters]
- FBP is a pontetial analgesic drug in neuropathic pain. [56 characters]