PRISCILA TIEMI KAWASHITA

O NÚCLEO ACCUMBENS E A SUBSTÂNCIA CINZENTA PERIAQUEDUTAL MODULAM DE MODO DISTINTO A HIPERALGESIA INFLAMATÓRIA CRÔNICA E AGUDA EM RATOS.

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do título de Mestre em Odontologia, Área de Fisiologia Oral

Orientadora: Prof.ª Dr.ª Adriana Pelegrini da Silva

Co-orientadora: Prof.ª Dr.ª Cláudia Herrera Tambeli

PIRACICABA

2008

FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecário: Sueli Ferreira Julio de Oliveira – CRB-8ª. / 2380

K179n

Kawashita, Priscila Tiemi.
O núcleo accumbens e a substância cinzenta periaquedutal modulam de modo distinto a hiperalgesia inflamatória crônica e aguda em ratos. / Priscila Tiemi Kawashita. -- Piracicaba, SP : [s.n.], 2008.

Orientador: Adriana Pelegrini da Silva
Dissertação (Mestrado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Dor. 2. Dinoprostona. 3. Hiperalgesia. 4. Sistema nervoso central. I. Silva, Adriana Pelegrini da . II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.
(sfjo/fop)

Título em Inglês: Periaqueductal gray matter and nucleus accumbens differently modulate chronic and acute hyperalgesia in rats
Palavras-chave em Inglês (Keywords): 1. Pain. 2. Dinoprostone. 3. Hyperalgesia. 4. Central nervous system.
Área de Concentração: Fisiologia Oral
Titulação: Mestre em Odontologia
Banca Examinadora: Adriana Pelegrini da Silva, Gisele Picolo, Fernanda Klein Marcondes
Data da Defesa: 27-02-2008
Programa de Pós-Graduação em Odontologia

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

A Comissão Julgadora dos trabalhos de Defesa de Dissertação de MESTRADO, em sessão pública realizada em 27 de Fevereiro de 2008, considerou a candidata PRISCILA TIEMI KAWASHITA aprovada.

PROFa. DRa. ADRIANA PELEGRINI DA SILVA

PROFa. DRa. GISELE PICOLO

PROFa. DRa. FERNANDA KLEIN MARCONDES

Dedicatória:

*A **Deus** por estar sempre presente me abençoando e guiando meu caminho.*

*Ao meu querido marido **Marcelo Teixeira Pombo** pela motivação desde o início, pela compreensão, respeito e apoio mesmo nos momentos mais difíceis.*

*Aos meus pais **Luiz** e **Graciela** pelo amor, confiança e pela minha formação como pessoa.*

*Aos meus irmãos **Cristina** e **Luiz Fernando** pela eterna e sincera amizade, incentivo e cumplicidade.*

***Agradecimentos especiais**:*

*À minha orientadora e amiga **Adriana Pelegrini da Silva**, pelo carinho, paciência, confiança e aprendizado.*

*À professora **Cláudia Herrera Tambeli** pelas preciosas orientações e suporte.*

*À professora **Maria Cecília Ferraz de Arruda Veiga** pela amizade e pelo exemplo como docente e pessoa.*

*À sempre amiga **Maria Cláudia Gonçalves Oliveira Fusaro** pelo exemplo, companherismo e inestimável apoio.*

Agradecimentos:

Ao Professor Dr. **Carlos Amílcar Parada** pelas orientações e valiosa colaboração em todas as etapas deste trabalho.

Ao Professor Dr. **Sérgio Roberto Peres Line** pela participação no processo de qualificação e por suas inteligentes dicas.

À professora Dr.ª **Fernanda Klein Marcondes** pelo aprendizado e pelo exemplo de dedicação à pesquisa.

Ao senhor **Carlos Alberto A. Feliciano** pelo suporte técnico e apoio diário. Às senhoras **Eliete Righetto**, **Maria Eliza dos Santos** e **Eliane Melo Franco** pela atenção e carinho.

Às amigas **Juliana Trindade Clemente Napimoga** e **Karla Elena Torres Chavez** pelo carinhoso acolhimento no início dos trabalhos no laboratório, pela paciência e amizade.

Às amigas **Juliana Maia Teixeira** e **Ana Paula Botelho** pelos ótimos momentos de convívio e cumplicidade.

A todos os meus amigos e colegas da pós-graduação pela alegria da convivência.

À Faculdade de Odontologia de Piracicaba FOP-UNICAMP por permitir a realização deste trabalho e por seus funcionários que, direta ou indiretamente, colaboraram com a finalização deste.

À **FAPESP** – Fundação de Amparo à Pesquisa do Estado de São Paulo pelo suporte financeiro para a realização deste trabalho.

"*Só sei que nada sei".*

Sócrates 470-399 a.C.

RESUMO

A modulação da dor pelo sistema nervoso central (SNC) consiste na inibição ou facilitação da excitabilidade do corno dorsal da coluna espinhal. O núcleo Accumbens (Nacc) e a Substância Cinzenta Periaquedutal (PAG) são duas importantes estruturas envolvidas na modulação da dor pelo SNC. A proposta deste estudo foi investigar o papel destas estruturas na modulação da hiperalgesia inflamatória aguda e persistente, induzida pela administração de Prostaglandina E_2 (PGE_2) na pata de ratos. A administração local subcutânea de PGE_2 induz um quadro de hiperalgesia que cede completamente em 24 horas. Entretanto, duas semanas de injeções intraplantares de PGE_2 induzem uma hiperalgesia que persiste por 30 dias após cessar o tratamento. Os resultados deste estudo demonstraram que a microinjeção no NAcc de lidocaína ou de cloreto de cobalto ($CoCl_2$), um bloqueador de canal de Cálcio, reduziu significativamente a hiperalgesia persistente, mas não modificou a hiperalgesia mecânica aguda induzida pela PGE_2 , medida tanto pelo teste de Randall-Selitto quanto pelo teste de Von Frey. Em contraste, a lidocaína ou o $CoCl_2$ injetados na PAG não modificaram a hiperalgesia persistente, mas aumentaram a hiperalgesia aguda induzida pela PGE_2 . Também demonstramos que a administração de L-Glutamato no NAcc restaurou a hiperalgesia persistente inibida pela administração local de Dipirona na pata. Estes resultados sugerem que o NAcc, mas não a PAG, está envolvido na manutenção da hiperalgesia persistente induzida pela PGE_2 e pode também participar na recorrência da dor crônica de origem inflamatória.
Palavras-chave: facilitação descendente, núcleo Accumbens, dor crônica, Substancia Cinzenta Periaquedutal.

ABSTRACT

The pain modulation by Central Nervous System (CNS) consists in inhibition or facilitation of the spinal dorsal horn excitability. The nucleus accumbens (NAcc) and periaqueductal gray matter (PAG) are two important structures involved in the pain modulation by CNS. The purpose of the present study was to investigate the role of NAcc and PAG on the modulation of the acute and persistent inflammatory hyperalgesia induced by prostaglandin E_2 (PGE_2) in rat. The local subcutaneous administration of PGE_2 induces hiperalgesia that is completely resolved in 24 h. However, 2 weeks of daily intraplantar treatment with PGE_2 induces hyperalgesia that persists for more than 30 days after the treatment cessation. The findings of this study demonstrated that the local injection of lidocaine or $CoCl_2$, a calcium channel blocker in the NAcc significantly reduced the persistent, but did not modify acute PGE_2-induced mechanical hyperalgesia, measured by either Randall-Sellito or Von Frey tests. In contrast, lidocaine or $CoCl_2$, injected in the PAG did not modify the persistent hyperalgesia, but increased the acute hyperalgesia induced by PGE_2. We also demonstrated that the administration of L-glutamate in NAcc restored the persistent hyperalgesia inhibited by the local administration of dipyrone in the hind paw. These results suggest that NAcc, but not PAG is involved in the maintenance of the PGE_2-induced persistent hyperalgesia and may also be implicated in the recurrence of chronic pain of inflammatory origin.
Keywords: descending facilitation, nucleus accumbens, chronic pain, PAG.

LISTA DE ABREVIATURAS E SIGLAS

CNS	Central Nervous System
AP	Antero-posterior
$CoCl_2$	Cloreto de Cobalto
COX	Cicloxigenase
H	Dorso-ventral
IASP	International Association for the Study of Pain
IL-1ß	Interleucina 1ß
i.m.	Intra-muscular
Intra-Acc	Intra-núcleo Accumbens
i.pl	Intra-plantar
i.p.	Intra-peritonial
L	lateral
Nacc	Núcleo Accumbens
PAG	Periaqueductal Gray Matter ou Substância Cinzenta Periaquedutal
PGE_2	Prostaglandina E_2
SNC	Sistema Nervoso Central
TNF-α	Fator de Necrose Tumoral α

SUMÁRIO

INTRODUÇÃO

A hiperalgesia é um sinal clássico da inflamação e, pelo menos em parte, resulta da sensibilização de nociceptores aferentes primários. Com base na ação analgésica das drogas anti-inflamatórias não esteroidais, os inibidores da COX, hoje se aceita que as prostaglandinas estão entre os principais fatores que contribuem para a hiperalgesia inflamatória (Ferreira, 1972; Ferreira *et al*., 1978).

Apesar da maioria dos mecanismos envolvidos na hiperalgesia aguda inflamatória ter sido elucidada nas últimas décadas, os estados de dor crônica continuam mal compreendidos e conseqüentemente com sucesso terapêutico limitado, o que pode ser devido, pelo menos parcialmente, ao escasso leque de drogas apropriadas para estes casos.

A falta de modelos experimentais que reproduzam fielmente o que ocorre com humanos acometidos por dores crônicas é uma preocupação constante. Modelos apropriados são fundamentais para estudar os mecanismos da dor crônica, especialmente para estudar os mecanismos envolvidos nesta cronicidade, que deve ocorrer em adição àqueles envolvidos na hiperalgesia inflamatória aguda. Há muitas causas para a dor crônica, mas certamente muitas delas estão usualmente associadas com episódios de inflamações agudas. (Aley *et al*., 2000).

Embora estímulos nociceptivos desencadeados pela formalina, carragenina ou pela incisão da superfície plantar sejam considerados persistentes, o seu pico de hiperalgesia não ultrapassa 3h após sua aplicação, e logo após desaparece. Ferreira *et al.* (1990) desenvolveram um modelo de hipernocicepção que, uma vez instalada, persiste por vários dias. Neste modelo, são aplicadas 14 injeções de Prostaglandina E_2 (PGE_2) na região intraplantar de ratos, uma por dia, e desta forma há a indução de um quadro hiperalgésico que perdura por mais de 30 dias após a cessação do tratamento. A fibra aferente primária parece adquirir "memória", pois a atenuação do quadro hipernociceptivo por uma injeção local de dipirona é rapidamente revertida por doses muito baixas de prostaglandina. Esta memória periférica pode explicar a fácil indução de períodos recorrentes de dor crônica (Ferreira *et al*., 1990).

Esta hiperalgesia persistente poderia mimetizar a dor inflamatória crônica em humanos, visto que uma vez administradas as citocinas pró-inflamatórias TNF-α ou IL-1β, que acabam por provocar a liberação de PGE_2, também há indução de hiperalgesia persistente da mesma magnitude e duração daquela induzida pela PGE_2 (Sachs *et al*., 2002).

Foi demonstrado que além da medula espinhal, outras estruturas do Sistema Nervoso Central estão envolvidas na modulação da dor aguda. Regiões supraespinhais, incluindo mesencéfalo, áreas talâmicas e corticais agem como estações de transmissão nociceptiva ascendente, mas também são consideradas como origem de vias modulatórias descendentes (Basbaum & Fields, 1984; Stamford, 1995; Willis & Westlund, 1997).

No estudo de Reynolds (1969), a estimulação elétrica da Substância Cinzenta Periaquedutal (PAG) possibilitou uma laparotomia em ratos sem a utilização de agente anestésico químico. A partir deste estudo clássico, caracterizou-se a existência de uma via descendente de controle inibitório da dor. A Substância Cinzenta Periaquedutal (PAG) é considerada a estação de retransmissão chave no processo da informação nociceptiva e antinociceptiva (Behbehani, 1995; Millan, 1999; Mitsui *et al*., 2003). A subdivisão ventrolateral da PAG produz exclusivamente antinocicepção em ratos (Fardin *et al*., 1984), e deprime a resposta de neurônios da medula espinhal frente a estímulos periféricos nociceptivos (Sandkuhler, 1996; Pelegrini-da-Silva *et al*., 2005). Adicionando-se aos clássicos centros antinociceptivos intrínsecos tais como PAG e Núcleo da Rafe, outras estruturas têm ganhado importância na modulação da dor. O Núcleo Pretectal, a Formação Reticular, o Núcleo do Trato Solitário e a Amígdala parecem também modular a informação nociceptiva (Prado & Roberts, 1985; Randich & Aicher, 1988; Hammond *et al*., 1992; Oliveira & Prado, 2001; Magdalena *et al*., 2004; Villarreal *et al*., 2004a; Knyihar & Csillik, 2006).

Mais recentemente, o Núcleo Accumbens (Nacc), núcleo mesolímbico relacionado ao processamento de informações motivacionais, especialmente à recompensa, também tem sido demonstrado como participante da modulação nociceptiva (Ma

et al., 1992; Neto *et al*., 1999; Magnusson & Martin, 2002; Taylor *et al*., 2003; Knyihar & Csillik, 2006). O Nacc parece ser um núcleo importante na inibição de uma atividade tônica de neurônios de projeção espino-supraespinhais, induzindo uma antinocicepção heterosegmentar (Gear & Levine, 1995; Gear *et al*., 1999; Tambeli *et al*., 2002; Tambeli *et al*., 2003). Apesar da modulação da dor aguda por estas estruturas estar bem documentada, sua influência na hiperalgesia inflamatória crônica ainda não está clara.

Considerando a importância da PAG, um componente chave no controle nociceptivo descendente (Behbehani, 1995; Millan, 2002), e o Nacc, uma estação de retransmissão da informação nociceptiva espinhal para o sistema mesolímbico (Gear & Levine, 1995), o objetivo deste estudo foi investigar como estas estruturas modulam a hiperalgesia inflamatória aguda e crônica. Para tal, foi medido o limiar mecânico da pata traseira de ratos onde foi induzida uma hiperalgesia inflamatória aguda ou crônica pela(s) injeção(ões) de PGE_2 e observou-se o efeito da inibição química da atividade da PAG ou do Nacc nesta hiperalgesia na pata. Posteriormente, em outro grupo experimental, instalou-se um quadro hiperalgésico crônico na pata e atenuou-se esta hiperalgesia com uma injeção local de Dipirona, provocando o que se denomina uma fase quiescente. Através da estimulação química do Nacc foi possível observar como esta estrutura do SNC parece modular este processo hiperalgésico persistente periférico que se encontrava em uma fase quiescente.

CAPÍTULO

O seguinte artigo será encaminhado para publicação no periódico Pain.

Periaqueductal Gray Matter and Nucleus Accumbens differently modulate chronic and acute hyperalgesia in rats.

Priscila Tiemi Kawashita1

Maria Cláudia Oliveira1

Cláudia Herrera Tambeli1

Carlos Amílcar Parada2

Adriana Pelegrini-da-Silva1*

1 Laboratory of Orofacial Pain, Department of Physiological Sciences, Piracicaba Dental School, University of Campinas - UNICAMP

2 Department of Physiology and Biophysics, Institute of Biology, University of Campinas - UNICAMP

* Corresponding author:

Limeira Av, 901 Zip Code: 13414-900 Piracicaba, São Paulo - Brazil

Tel / Fax: + 55-19-21065212

E-mail address: adriana-ps@bol.com.br (Pelegrini-da-Silva A.)

Abstract

Pain modulation by Central Nervous System (CNS) consists in inhibition or facilitation of the spinal nociceptive activity. Periaqueductal Gray Matter (PAG) and Nucleus Accumbens (NAcc) are two important structures involved in the pain modulation by CNS. The purpose of the present study was to investigate the role of PAG and NAcc in the modulation of acute and persistent hyperalgesia induced by prostaglandin E_2 (PGE_2) in rat. The hind paw subcutaneous administration of PGE_2 induces hyperalgesia that is completely resolved in 24 h. However, two weeks of daily intraplantar treatment with PGE_2 induce hyperalgesia that persists for more than 30 days after the treatment cessation. The microinjection of lidocaine or the calcium channel blocker $CoCl_2$ in the PAG did not modify significantly the magnitude of persistent hyperalgesia and allodynia measured respectively by Randall-Sellito and Von Frey test. In contrast, the blockade of PAG activity by lidocaine increased the acute hyperalgesia and acute mechanical allodynia. In the NAcc, the microinjection of lidocaine or $CoCl_2$ significantly reduced the persistent hyperalgesia and allodynia, while increased the acute mechanical hyperalgesia and had no significant effect on acute allodynia. Furthermore, the NAcc neuronal activation by local microinjection of L-glutamate restored the magnitude of the persistent hyperalgesia, which had been previously reduced by the hind paw administration of dipyrone. These results suggest that PAG and NAcc modulate differently acute and persistent hyperalgesia and allodynia. In addition, the NAcc, but not PAG seems to be involved in the

maintenance of the PGE_2-induced persistent hyperalgesia and allodynia and may also play a role in recurrent episodes of pain in chronic painful states. Keywords: descending facilitation, nucleus accumbens, chronic pain, PAG, PGE_2, hyperalgesia.

1. Introduction

Hyperalgesia is a classical sign of inflammation and results, at least in part, from the sensitization of primary afferent nociceptors. Based on the analgesic action of non-steroidal anti-inflammatory drugs, i.e., cyclooxygenase inhibitors, it is well accepted that prostaglandins are some of the main contributors to inflammatory hyperalgesia [13, 15].

Although many of the mechanisms underlying acute inflammatory hyperalgesia have been elucidated in the last decades, chronic pain physiopathology remains poorly understood with limited therapeutic success, possibly due to the small range of available drugs. The lack of experimental models with a good degree of human therapeutic predictability may be one important concern in chronic pain studies. In the rat model of persistent mechanical hyperalgesia [14] the sensitization of the rat hind paw lasts for more than 30 days following the cessation of 14 successive daily intraplantar injections of prostaglandin E_2 (PGE_2). This model may be useful to study the mechanisms of chronic hyperalgesia, which may occur in addition to those involved in acute hyperalgesia.

Supraspinal regions, i.e. periaqueductal gray matter (PAG) and raphe nucleus are involved in the modulation of acute pain [5, 61, 76]. The PAG is a key relay station in the processing of nociceptive and antinociceptive information [6, 41, 44]. The ventrolateral PAG subdivision produces exclusively antinociception in

rats [12], and depresses the response of spinal cord neurons to peripheral noxious stimulations [52, 59].

The nucleus accumbens (NAcc), which is known to be an important component of the mesolimbic reward system and implicated in substance abuse, also plays a role in pain modulation, as evidenced by the antinociception induced by direct microinjection of opioid into the NAcc core [33, 66, 77]. NAcc also participates in a novel ascending nociceptive control circuit [19], where an intense chemical or thermal noxious stimulation induces pain modulation by an ascending nociceptive control dependent on opioid and dopamine links in the nucleus accumbens[18, 19, 63, 64].

Although it is well known that structures of the CNS modulate acute pain, whether they also modulate chronic hyperalgesia remains to be investigated. Therefore, the aim of this study was to investigate whether PAG and NAcc modulate acute and persistent hyperalgesia. To address this issue, the effect of the inhibition of the activity of PAG or NAcc was tested on the acute and persistent mechanical hyperalgesia and allodynia induced by PGE_2.

2. Materials and Methods

2.1 Subjects and surgery

We used male albino Wistar rats weighing 200-300 g. The experiments were conducted in accordance to the International Association for the Study of Pain guidelines on using laboratory animals [83] and all experimental procedures and protocols were previously approved by the Committee on Animal Research of the University of Campinas. The animal discomfort and the number of animals per group were kept to a minimum. Rats were housed in plastic cages with soft bedding (five per cage) on a 12-h light/dark cycle (lights on at 06:00 AM) with food and water available *ad libitum*. They were maintained in a temperature-controlled room (23 °C) and handled for at least one week before the experiments.

Rats were anesthetized with Xylazine Chloride (10 mg/kg) and Ketamine Hydrochloride (90 mg/kg) and a 15 mm length of a 23 gauge stainless-steel guide cannula was stereotaxically implanted into the skull until its tip reach 3 mm above the NAcc core or ventrolateral PAG. According to the atlas of Paxinos & Watson (1986), the coordinates (in mm) for ventrolateral PAG were AP +0.5 (relative to the lambda), L -2 (relative to the sagittal suture), H -6.5 (relative to the skull surface); and for NAcc core were AP +1.3 (relative to Bregma), L $\pm$1.8 (relative to the sagittal suture), H – 7.2 (relative to the skull surface). The guide cannula was fixed on the skull with one steel screws and dental cement. After the surgical procedure,

rats received penicillin (50 mg/kg, i.m.) and recovered for at least 1 week before the experiments.

2.2 Behavioral Nociceptive tests

The nociceptive mechanical threshold was measured by Randall-Sellito analgesimeter and Von Frey filaments (Stoelting, Wood Dale, IL, USA) to determine hyperalgesia and allodynia respectively. The Randall-Sellito nociceptive paw-withdrawal flexion reflex test [57] was performed using an Ugo-Basile analgesymeter (Stoelting, Chicago, IL, USA), which applies a linearly increasing mechanical force (in grams) to the plantar surface of the rat's hind paw [2]. The basal paw-withdrawal threshold was defined as the mean of three measures performed at 5-min intervals before the first intraplantar injection of PGE_2. The variation (Δ) of mechanical hyperalgesia expressed on the graphs were calculated by subtracting the mean of three consecutive measurements after the treatment evaluated (test) from the basal threshold (Δ of mechanical hyperalgesia = basal – test).

For the Von Frey test, the paw was touched with one of a series of filaments with logarithmically incremental stiffness (0.008 – 15 g, lower and upper limit of the test, respectively). Each filament was applied vertically to the plantar surface of the rat paw with sufficient force to bend slightly the filament. A single trial consisted of six applications of a particular filament, applied once every 3–4 s.

Testing was initiated with the 2.04 g filament in the middle of the series. A response was defined as a sharp withdrawal of the stimulated paw. The up–down method was used to record the threshold [8]. The upper limit value (15 g) was recorded if there was no withdrawal response to this force.

2.3 Microinjection procedures

Drugs or saline were microinjected in the NAcc or the PAG using a 30 gauge stainless-steel needle. The needle was inserted into the guide cannula immediately before microinjection and advanced to protrude 3 mm beyond the guide cannula tip. The volume of each injection was 0.25 μl delivered at a constant rate over a period of 1 min. The needle was removed 30 s after the completion of the drug administration.

2.4 PGE_2-induced acute and persistent hyperalgesia.

PGE_2 or saline was administrated in the subcutaneous tissue of the plantar surface of rat's right hind paw by a 30-gauge needle connected to a catheter of polyethylene coupled to a Hamilton syringe (50 μl). Rats were briefly restrained and the volume of injection was 50 μl.

The dose of PGE_2 used in this study (400 ng) was the lowest dose obtained from a dose-response curve, previously determined, that evoked the maximum

acute decrease in mechanical threshold tested by Randall-Sellito or Von Frey test 3 h after its administration.

As previously described [14], two weeks of daily local subcutaneous administration of PGE_2 induce persistent mechanical hyperalgesia that remains for more than 30 days after the discontinuation of the PGE_2 treatment. All animals were treated with i.p. administration of indomethacin (2 mg/kg) 30 min before PGE_2 injections to avoid endogenous release of prostaglandins related to the injections trauma.

2.5 Histology

At the end of the experiment, pontamine blue dye (0.25 μl) was microinjected to label the sites of intracerebral injection. Rats were sacrificed with an overdose of urethane and perfused through the heart with saline followed by buffered formalin–saline solution. Dye spots were localized on 40 μm serial brain coronal sections, and identified on diagrams from the atlas of Paxinos & Watson (1986). Only rats showing dye spots in the target structure were considered for further analysis.

2.6 Drugs

The following drugs were used: 2% lidocaine [62]; prostaglandin E_2 400ng/paw [73]; L-glutamic acid hydrochloride 6.9 ng/ 0.25µl [72]; Indomethacin, 2.0 mg/Kg [26]; dipyrone 240 µg/ paw [14], cobalt chloride 1.3 ng/ 0.25 µl [31]. All drugs were obtained from Sigma Chemicals, St. Louis, MO, USA and dissolved in phosphate-buffered saline [PBS; 20mM sodium phosphate buffer, pH 7.4, containing 0.9% (weight/volume) sodium chloride].

2.7 Experimental Design

A guide-cannula was bilaterally stereotaxilcally implanted in the ventrolateral PAG or Nacc core one week before the day of acute and chronic experiments.

On the day of the acute experiment, we measured the mechanical threshold before and 3 hours after acute i.pl. PGE_2 or saline injection. For the chronic hyperalgesia, the mechanical threshold was measured before the first injection of PGE_2 or saline and 6 days after the disruption of the successive injections to confirm the installation of persistent hyperalgesia. After that, rats received intra-PAG or intra-NAcc microinjections of 2 % lidocaine, saline or 1.3 ng/ 0.25 µl $CoCl_2$. Fifteen minutes later the mechanical threshold was measured again. $CoCl_2$ is an

inorganic blocker of the calcium channel conductance and therefore utilized here to impair synaptic transmission [31].

In another series of experiments, L-glutamate (6.9 ng/ 0.25µl) was administrated in the NAcc three days after locally i.pl. administration of dipyrone (240 μg/ paw in 50 μl) in rats with persistent hyperalgesia. The dose of dipyrone was previously established by a dose-response curve and did not elicit a systemic effect, as evidenced by the absence of analgesic effect when injected on the paw contralateral to the PGE_2 injected paw (data not shown).

2.8 Statistical analysis

The Von Frey results were analyzed as medians with their corresponding confidence limits (95%). The experimental groups were compared by the non-parametric Kruskal–Wallis or Mann-Whitney test. Multiple comparisons after the Kruskal–Wallis test were performed using the two-tailed Mann–Whitney test. For repeated measures, we used the Wilcoxon matched paired test. For the Randall-Sellito test, one-way ANOVA or unpaired t test was performed. For repeated measures, we used Repeated Measures ANOVA. If there was a significant between treatments, post-hoc contrasts, using the Tukey test, were performed to determine the basis of the significant difference. Data of all groups are expressed as means ± S.E.M and the level of significance was set at $P< 0.05$.

3. Results

3.1 PGE_2 -induced acute and persistent hyperalgesia

A single i.pl. injection of PGE_2 (400 ng) induced acute mechanical hyperalgesia and allodynia 3h after its injection when compared with the saline control group, measured by Randall-Sellito (Fig. 1A, Mann-Whitney test, p= 0.02) and Von Frey test (Fig. 1B, T test, p<0.0001) respectively. The acute hyperalgesia and allodynia were completely resolved 24 h after PGE_2 administration (data not shown). Rats treated with PGE_2 during 14 days presented, six days after the disruption of the PGE_2 treatment (in the 20^{th} day), a persistent hyperalgesia and allodynia when compared with the control group, measured by Randall-Sellito (Fig. 1C, p=0.0019, Mann-Whitney test) and Von Frey test (Fig. 1D, p<0.0001, T test). The inset in figure 1D shows the progression of mechanical threshold reduction, measured by Von Frey test before and 3 h after each diary i.pl. injection of PGE_2.

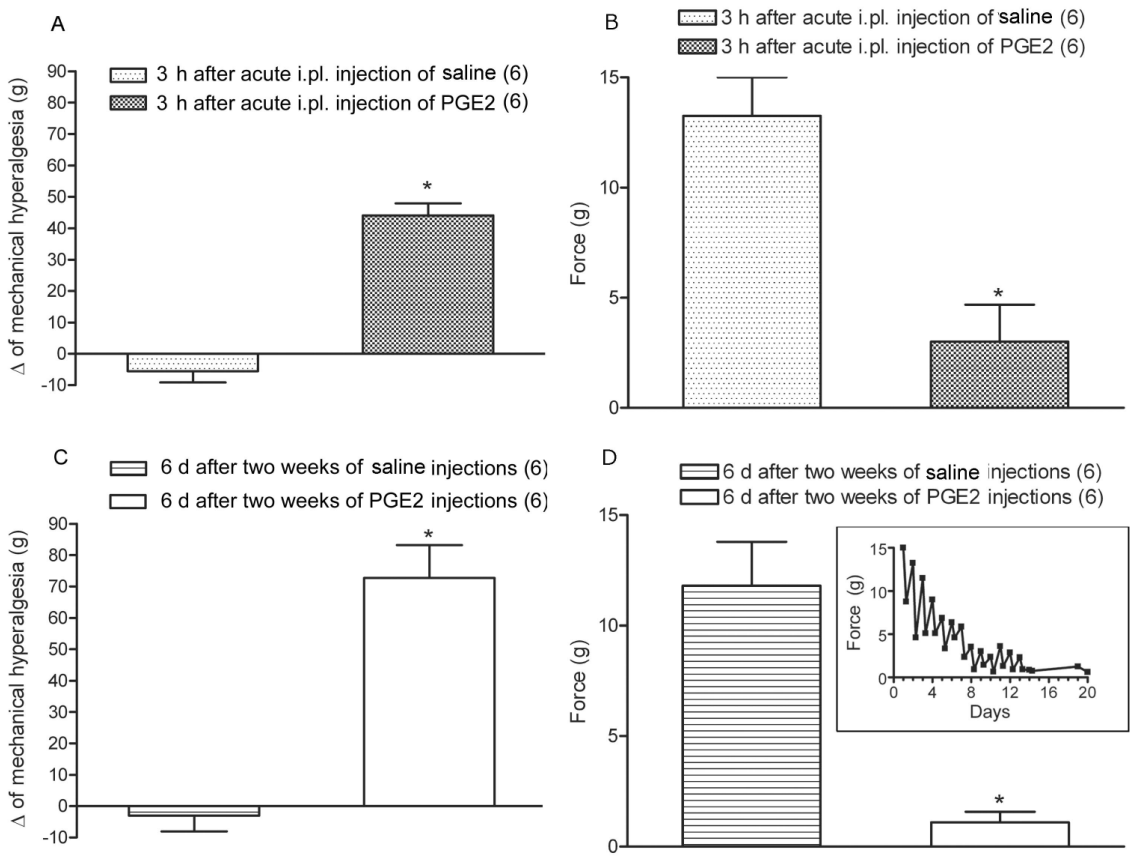

Figure 1: Induction of acute and persistent hyperalgesia and allodynia by i.pl. PGE_2 injection. Mechanical hyperalgesia (A) and allodynia (B) induced by a single i.pl. injection of PGE_2 (400 ng/ 50 µl) or saline, measured by Randall-Sellito (t test) and Von Fey test (Mann-Whitney test) respectively. Persistent hyperalgesia (C) and allodynia (D) induced by fourteen days of daily injection of PGE_2 (400 ng/ 50 µl). The data represent the behavioral mechanical response at the 6th day after the last PGE_2 or saline injection. The symbol (*) indicates a significant increase in the mechanical hyperalgesia or allodynia in relation to the controls. The daily mechanical threshold measured by Von Frey hair filaments before and 3h after each injection of PGE_2 is represented in the inset in figure D.

For this and subsequent figures each bar represents the mean ± SEM. Animal number per group is expressed in parenthesis. $P<0.05$.

3.2 Periaqueductal gray matter modulated acute but not persistent hyperalgesia

Only rats with microinjections into the ventrolateral PAG or NAcc core were included in data analysis (Fig. 2). Injection sites outside the boundaries of the ventrolateral PAG and NAcc core were not included in data analysis.

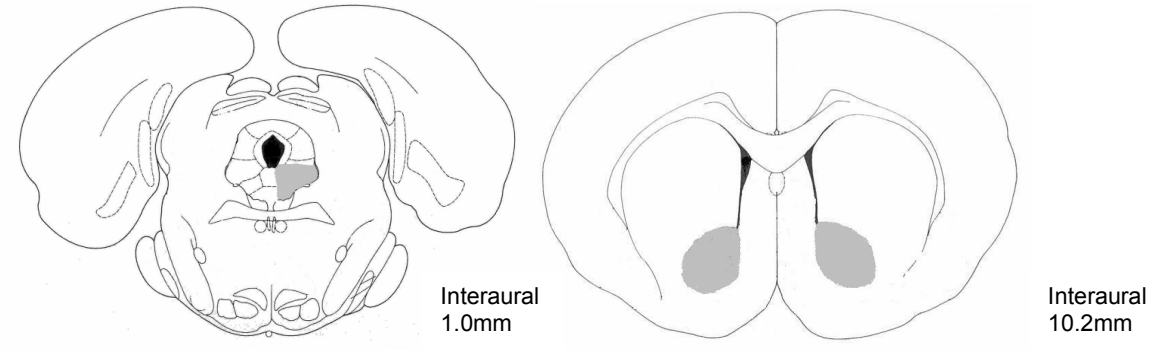

Figure 2: Location of microinjection sites in the ventrolateral PAG and Nacc core for all experiments. Only Lidocaine, $CoCl_2$, L-Glutamate and saline injections within the shaded regions were included in data analysis. Coronal planes were taken from the Atlas of Paxinos and Watson (1986) and the number in the right refers to the distance from the interaural line. All injections fell within coronal planes distant 1.0 to 1.36 mm for PAG and 9.48 to 10.7 mm for Nacc from the interaural line.

The microinjection of 2% lidocaine into the PAG 3h after a single i.pl. injection of 400 ng PGE_2 induced a significant increase in the mechanical hyperalgesia and allodynia compared to the PAG-saline microinjection, as measured by Randall-Sellito (Fig. 3A, T test) and Von Frey test (Fig. 3C, Mann-Whitney test) respectively. In contrast, the injection of lidocaine or $CoCl_2$ (1.3 ng/ 0.25 µl) into

the PAG had no significant effect on the magnitude of persistent hyperalgesia (Fig. 3B, Tukey test) and allodynia (Fig. 3D, Kruskall-Wallis test).

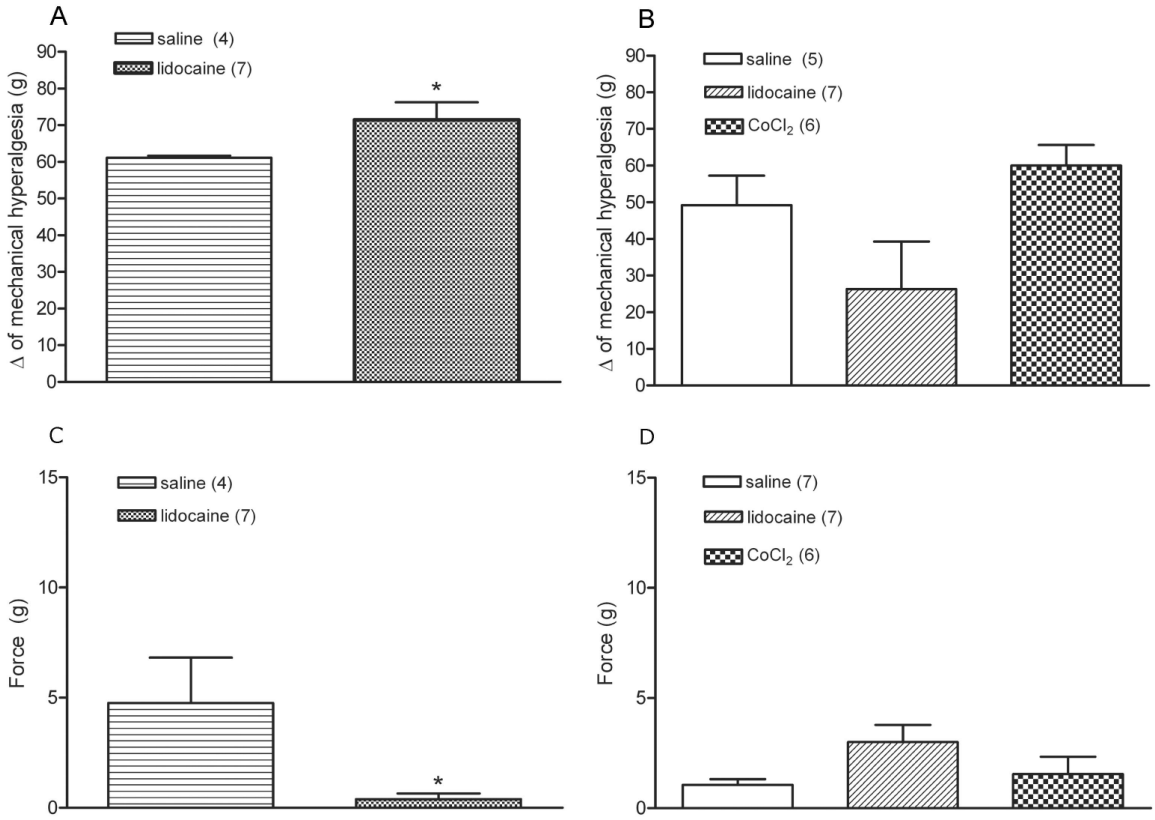

Figure 3: Modulatory effect of the blockade of the PAG neuronal activity on acute and persistent hyperalgesia and allodynia. Mechanical hyperalgesia (A) and allodynia (C) observed after a microinjection of 2 % lidocaine (0.25 µl) or saline in the PAG of rats receiving a single i.pl. injection of PGE2 (400 ng/ 50 µl), measured by Randall-Sellito (t test) and Von Frey test (Mann-Whitney test), respectively. Persistent hyperalgesia (B) and allodynia (D) observed after a microinjection of 2% lidocaine, saline or CoCl2 (1.3 ng/ 0.25 µl) into the PAG of rats, 6 days after the two weeks of i.pl. injections of PGE2, measured by Randall-Sellito (Tukey test) and Von Frey test (Kruskall-Wallis test), respectively.

The symbol (*) indicates hyperalgesia significantly higher than that observed in the control.

3.3 Nucleus Accumbens inhibited acute and facilitated persistent hyperalgesia

The bilateral microinjection of 2% lidocaine (0.25µl) but not saline into the NAcc core increased significantly the acute hyperalgesia, measured 3h after a single i.pl. injection of PGE_2 (400 ng) (Fig. 4A, T test) and decreased significantly the persistent hyperalgesia (Fig. 4B, Tukey test), measured by Randall-Sellito test. In the Von Frey test, the acute allodynia measured 3h after the i.pl injection of PGE_2 (400 ng) was not significantly modified by microinjection of 2% lidocaine in the bilateral NAcc (Fig. 4C, Mann-Whitney test). However, the magnitude of persistent allodynia was significantly decreased by the lidocaine microinjection in the NAcc (Fig. 4D, Kruskall-Wallis test). The NAcc microinjections of $CoCl_2$ (1.3 ng / 0.25 µl) also decreased the magnitude of the persistent hyperalgesia (Fig. 4B, $p=0.02$, Tukey test) and allodynia (Fig. 4D, Kruskall-Wallis test) in relation to the groups receiving NAcc microinjections of saline. However, the reduction in the mechanical threshold induced by the microinjection of $CoCl_2$ was significantly lower than that induced by the microinjection of lidocaine in both tests ($p<0.05$). In rats treated with daily i.pl. injection of saline, the bilateral microinjection of lidocaine into the NAcc did not induce analgesic effect compared to the rats receiving NAcc microinjection of saline as evidenced by Randall-Sellito (inset Fig. 4 B) and Von Frey test (inset Fig. 4D).

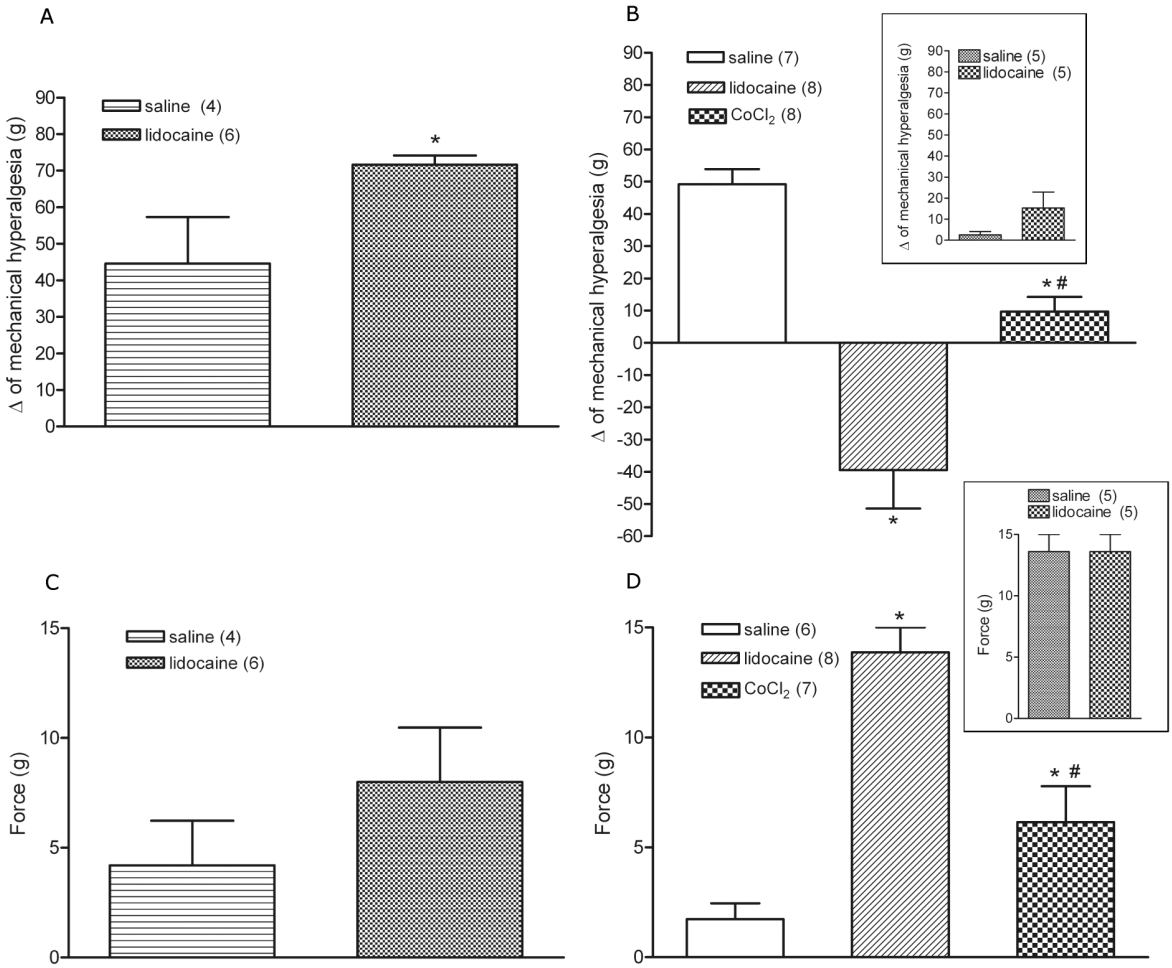

Figure 4: Modulatory effect of the blockade of neuronal Accumbens activity on the acute and persistent hyperalgesia and allodynia. (A) Acute mechanical hyperalgesia after the bilateral microinjection of 2% lidocaine (0.25 µl) or saline into the NAcc of rats receiving a single i.pl. injection of PGE_2 (400 ng/50µl, i.pl.), measured by Randall-Sellito test (t test). (B) Persistent hyperalgesia measured after the bilateral microinjection of 2% lidocaine, saline or $CoCl_2$ (1.3 ng/ 0.25 µl) on the NAcc of rats at the 6th day after the two weeks of i.pl. injection of PGE_2 (Tukey test). (C) Acute mechanical allodynia after the bilateral microinjection of 2% lidocaine (0.25 µl) or saline into the NAcc of rats receiving a single i.pl. injection of PGE_2 (400 ng/50µl, i.pl.), measured by Von Frey test (Mann-Whitney test). (D) Persistent allodynia measured after the bilateral microinjection of 2% lidocaine, saline or $CoCl_2$ (1.3 ng/ 0.25 µl) on the NAcc of rats at the 6th day after the two

weeks of i.pl. injection of PGE_2 (Kruskall-Wallis test). The graphs shown in the insets represent the mechanical hyperalgesia (B) and allodynia (D) of rats treated with two weeks of i.pl. injection of saline receiving at the 6^{th} after the last injection, a bilateral microinjection of lidocaine or saline into the NAcc, measured respectively by Randall-Sellito and Von Frey test.
The symbol (*) indicates hyperalgesia or allodynia significantly different from that observed in rats receiving NAcc microinjection of saline for all groups. The symbol (#) indicates hyperalgesia or allodynia significantly different from that observed in rats receiving NAcc microinjection of lidocaine.

3.4 Chemical activation of the NAcc reversed the dipyrone-dependent reduction on the persistent hyperalgesia

A single i.pl. injection of dipyrone (240 µg/ 50 µl/ paw) in the paw ipsilateral to that receiving two weeks of PGE_2 injections induced a significant reduction in the magnitude of the persistent mechanical hyperalgesia and allodynia, measured by Randall-Sellito (Fig. 5A, $p<0.0001$, Tukey test) and Von Frey test (Fig. 5C, $p=0.03$, Wilcoxon test). The microinjection of L-glutamate (Glu, 6.9 ng/ 0.25µl) into the bilateral NAcc restored the hyperalgesic state to the pre-dipyrone values in the Randall-Sellito and Von Frey test (Fig. 5A and C, respectively). One and two days after the injection of glutamate in the NAcc, the magnitude of the mechanical hyperalgesia was still elevated (Fig. 5A, $p<0.001$, Tukey test; Fig. 5C, $p=0.03$, Wilcoxon test). The NAcc microinjection of saline had no significant effect on the analgesic response elicited by i.pl. injection of dipyrone (240 µg/ 50µl), measured by Randall-Sellito (Fig. 5B, $p>0.05$, Tukey test) and Von Frey test (Fig. 5D, $p>0.05$, Wilcoxon test). The reduction in the intensity of persistent hyperalgesia

induced by dipyrone injection persisted at least for 4 days as can be observed in the Figs 5B and D.

For the Randall-Sellito test, the basal measurements were made before the 1st PGE_2 injection for all groups and the test values were taken 6 days after the end of the daily PGE_2 injections, after the dipyrone, L-glutamate or saline injections.

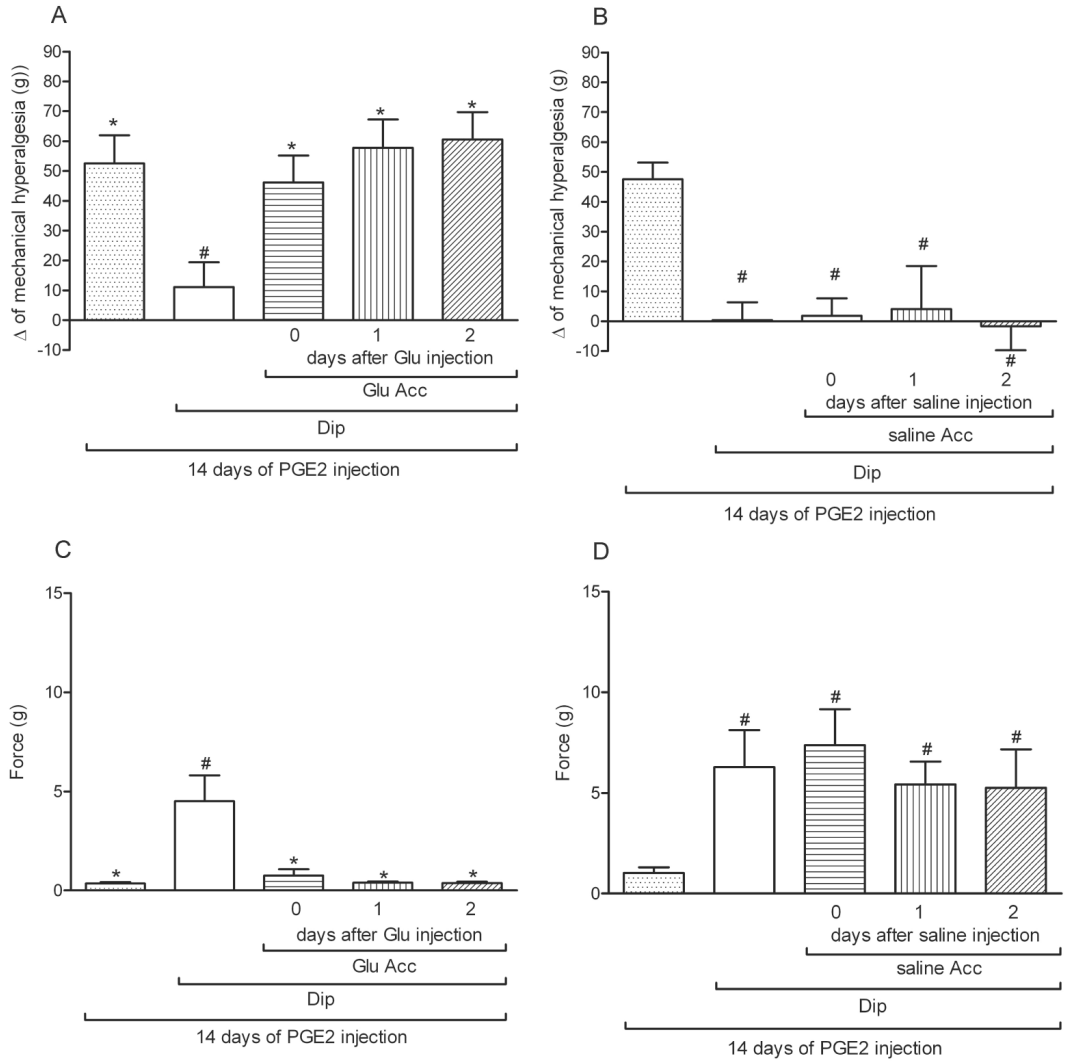

Figure 5: Modulatory effect of the activation of NAcc by the L-glutamate on the magnitude of the persistent hyperalgesia reduced by i.pl. injection of dipyrone. Mechanical hyperalgesia observed with bilateral NAcc microinjection of L-glutamate (6.9 ng/ 0.25µl) (A) or saline (B) performed 3 days after the i.pl. injection of dipyrone (240 µg/ 50 µl) in rats with persistent hyperalgesia induced by two weeks of daily i.pl. injection of PGE_2 (400 ng/ 50 µl), measured by Randall-Sellito test (Tukey test). Mechanical allodynia observed with bilateral NAcc microinjection of L-glutamate (6.9 ng/ 0.25µl) (C) or saline (D) performed 3 days after the i.pl. injection of dipyrone (240 µg/ 50 µl) in rats with persistent hyperalgesia induced by two weeks of daily i.pl. injection of PGE_2 (400 ng/ 50 µl), measured by Von Frey test (Wilcoxon test) (#) indicates significant reduction of the persistent hyperalgesia in relation to mechanical response measured at the 6^{th} day after the last PGE_2 injection and before de dipyrone injection. (*) indicates hyperalgesia significantly higher than the hyperalgesia measured 3 days after the i.pl. injection of dipyrone.
Abbreviations: Glu = L-glutamate; Dip = dipyrone.

4. Discussion

The current study demonstrated that PAG and NAcc modulate differently acute and chronic pain. We observed here an inhibitory activity of the PAG on the intensity of acute hyperalgesia and allodynia, while PAG has no significant effect on the modulation of persistent pain. NAcc also exerts an inhibitory influence on the acute hyperalgesia, contributing to the reduction of the hyperalgesia intensity. In contrast, a facilitatory influence from NAcc seems to contribute to the maintenance of the persistent hyperalgesia and allodynia. In addition, we observed that the neuronal activation of the NAcc is able to restore the magnitude of the hyperalgesia and allodynia previously reduced by a peripheral analgesic as dipyrone. Based on these observations, we suggested a new conception on the

role of the endogenous descending nociceptive control in the physiopathological mechanism of persistent pain that is the active participation of NAcc on the maintenance of the persistent hyperalgesia. Furthermore, NAcc and PAG seem to exert inhibitory, facilitatory or a non-significant influence on the hyperalgesia intensity depending on the duration of the pathology.

It is well-known that the antinociceptive descending control from the PAG and other brainstem structures are important to counteract the nociceptive activity in animal models of tonic noxious stimulation as the inflammatory [67], neuropathic [45, 54] and incisional pain [52, 72]. We observed here that in the acute hyperalgesia, induced by a single i.pl. injection of PGE_2, the injection of lidocaine in the PAG induced an increase in the mechanical hyperalgesia and allodynia, confirming that this nucleus exerts an inhibitory influence on the acute hyperalgesic states.

Usual animal models of tonic hyperalgesia are dependent on a single injection of inflammatory agents. Recently it has been developed a model of hyperalgesia that persists beyond the cessation of repetitive injections of PGE_2 [14], indicating the establishment of a neuronal memory for the nociceptive activity. Because the descending modulatory system undergoes time-dependent changes following persistent inflammation [9, 46, 82], an inhibitory or facilitatory activity of this system can predominate according to the progression of the hyperalgesia. In fact, in contrast to the antinociceptive activity on the acute hyperalgesia, we observed here that the blockade of neuronal activity of the PAG by the injection of

lidocaine or $CoCl_2$ had no significant effect on the magnitude of the persistent mechanical hyperalgesia and allodynia induced by the two weeks of i.pl. injections of PGE_2. It has been reported that the suppression or facilitation of nocifensive behavior and spinal dorsal horn hyperexcitability [9, 10, 60, 69, 81, 82] are modulated by encephalic structures. Thus, the intensity of perceived pain and hyperalgesia is fine-tuned by descending pathways. The imbalance of these modulating systems may be one mechanism underlying variability in acute and chronic pain conditions [25, 75]. Therefore, the absence of PAG inhibitory activity on the magnitude of the persistent mechanical hyperalgesia could contribute to the intensity and/or maintenance of the hyperalgesic states.

The activation of the descending control from the PAG differently modulates the wide-dynamic range responses to the myelinated vs. unmyelinated nociceptors activation [38, 39]. This characteristic explain, for example, the more intense control from the PAG to heat than to mechanical evoked responses [20, 54, 80]. Since the sensitizing action of the PGE_2 has been related to multiple classes of cutaneous nociceptors including C-polymodal, C-mechano-heat, C-mechano-cold nociceptors and A-delta high-threshold mechanoceptors [37], a daily schedule of PGE_2 injection could convey different modalities of pain. Therefore, the absence of PAG modulation of the persistent mechanical hyperalgesia does not exclude a possible involvement of this nucleus in the control of other modalities of stimuli, as heat-evoke withdraw reflexes.

Pain processing is a multidimensional experience that integrates several limbic and cortical structures to coordinate the behavioral response to nociceptive stimulation. The NAcc has been considered of particular interest in relation to pain [1, 18, 19, 24]. We showed here that the injection of lidocaine into the bilateral NAcc increased the hyperalgesia induced by a single injection of PGE_2, as measured by the Randall-Sellito test, but had no effect on the allodynia measured by Von Frey filaments, indicating that NAcc exerts an inhibitory influence on the acute hyperalgesia. Mechanical allodynia and hyperalgesia are likely to be mediated by different neuronal pathways [27, 40, 48, 74], what could explain the detection of the inhibitory influence from the NAcc to the acute hyperalgesia by the Randall-Sellito, but not by Von Frey test.

It has been well documented that NAcc exerts an inhibitory nociceptive activity. The dopamine [1, 65], N-methyl-D-aspartate [42], opioid peptides [1, 77] and neurokinin [3] are mediators involved in this antinociceptive action of the NAcc. Moreover, the NAcc plays an important role in the “mesolimbic loop” [24, 78] and heterosegmental analgesia [18, 19, 64]. Besides the antinociceptive activity of the NAcc on the acute hyperalgesia also observed here, the injection of lidocaine or $CoCl_2$ into the bilateral NAcc reduced the hyperalgesia and allodynia induced by the multiples injections of PGE_2, measured by Randall-Sellito and Von Frey test respectively. Since $CoCl_2$ blocks the calcium channel conductance [31], we conclude that reduction on the persistent hyperalgesia by $CoCl_2$ depends on the blockade of activity of neuronal cells in the NAcc, indicating that the

endogenous activity of the NAcc is facilitating the intensity of persistent pain. The greater reduction of mechanical hyperalgesia induced by NAcc microinjection of lidocaine compared to the $CoCl_2$ could represent an inhibition of the locomotor activity by the local anesthetic. However, the injection of lidocaine into the bilateral NAcc in rats treated with two weeks of i.pl. injections of saline did not reduce the basal mechanical threshold in the Randall-Sellito or Von Frey test, excluding the impairment of locomotor behavior by the injection of lidocaine in the NAcc. Therefore, the greater analgesic effect observed with the injection of lidocaine into the NAcc might be related to the additional blockade of fibers en passant originated in other nuclei with facilitatory nociceptive activity. In our knowledge, this is the first time that the NAcc is associated with the facilitatory control of the hyperalgesia.

In the model of PGE_2-induced persistent hyperalgesia used here, a single dose of dipyrone administrated in the site of PGE_2 treatment can induce a maintained reduction on the intensity of hyperalgesia. Once the hyperalgesia is reduced, a small dose of PGE_2, dopamine or Interleukin-1 beta, which in normal animals causes a mild and short lived effect, restored the previous magnitude of the hyperalgesia, characterizing an ease induction of recurrent periods of nociception [14]. In addition to our results indicating that NAcc is involved in the maintenance of persistent hyperalgesia, we also tested if NAcc could be involved in the recurrence of the hyperalgesic episodes in chronic pain. We observed here that the injection of L-glutamate into the bilateral NAcc reversed the dipyrone-

dependent reduction of the intensity of the persistent hyperalgesia and allodynia. Glutamate, which exerts excitatory effects on neuronal cell bodies and dendrites [16, 32], has been used earlier to evoke antinociception from several brain structures including the ventral portion of the PAG [6, 28, 68]. Since glutamate excites neuronal cell bodies but not fibers [16, 32], we conclude that the increase in the mechanical hyperalgesia and allodynia induced by L-glutamate injection on the NAcc depends on the activity of local neuronal cells, indicating that NAcc activation, in persistent hyperalgesic condition, can play a key role on the recurrence of the chronic pain.

The contribution of NAcc on the reestablishment of persistent hyperalgesia observed here could be dependent on the activity of pronociceptive pathways from the central nervous system in response to neuroplastic adaptations to persistent pain input [55, 67 69]. In addition, it is known that chemical activation of the spinal cord seems to induce a retrograde sensitization of the nociceptor [50], which would contribute to the peripheral hyperalgesia. Although this retrograde sensitization has been attributed to the release of nociceptive mediators in the spinal cord during the activation of primary afferent fibers [50], we can not exclude a pronociceptive action of descending fibers on the excitability of primary afferent fibers at the spinal cord level. Taken together, these observations point out for a possible participation of the NAcc on the maintenance and recurrence of the hyperalgesic state in chronic painful conditions. Although the NAcc contributes to the inhibitory control of nociception in models of acute and inflammatory pain [3,

21, 65], the predominance of a facilitatory activity of NAcc on the persistent hyperalgesia agrees with various pathological conditions where marked plastic changes in the descending control of spinal neurons are related to the duration of the processes [29, 53, 54, 69]. For example, a peripheral inflammatory process may reverse the rostral ventromedial medulla (RVM) induced inhibition of spinal dorsal horn neurons activity to a descending facilitation [9, 22]. In addition, some spinal neuroplastic changes associated with peripheral nerve injury, such as up-regulation of dynorphin, also depend upon descending facilitatory influences from the RVM [7, 17, 70].

Besides the recognized descending modulatory nociceptive pathway, it has been also described the existence of an antinociceptive path mediated by opioids ascending from the spinal cord to the NAcc [18, 19, 63, 64]. However, as the mechanical hyperalgesic response was determined here by flexion withdraw reflex, any variation in this response must be dependent on a final influence on the spinal cord neurons. Therefore, the descending facilitatory activity initiated in the NAcc during persistent hyperalgesia might also use a relay station before reaching spinal cord. However, since the blockade of PAG activity had no significant effect on the persistent hyperalgesia intensity, NAcc must use a different relay nucleus to the described PAG station [11, 34, 79] in the chronic hyperalgesic conditions.

In summary, this study demonstrated that descending endogenous nociceptive modulation seems to exert inhibitory or facilitatory influence on the hyperalgesia and allodynia depending on the acute or chronic condition of the pain

state. We also demonstrated for the first time the facilitatory activity of NAcc, contributing to the maintenance of the persistent hyperalgesia and the capability of the NAcc activation to restore the intensity of the chronic hyperalgesia. The relationship between our findings and the clinical chronic pain is not clear yet. However, it reinforces a previously suggestion that once initiated, maintenance of the spinal dorsal horn sensitization requires descending pain facilitatory mechanisms [7, 30, 49, 70, 75] and adds a new conception by suggesting that the NAcc can have an active participation on the recurrence of pain episodes.

Acknowledgments

This work was supported by grants from FAPESP and CNPq, Brazil. We thank Carlos Alberto Feliciano for excellent technical assistance.

References

[1] Airavaara M, Mijatovic J, Vihavainen T, Piepponen TP, Saarma M, Ahtee L . In heterozygous GDNF knockout mice the response of striatal dopaminergic system to acute morphine is altered. Synapse 2006;59:321-329.

[2] Aley KO, Martin A, McMahon T, Mok J, Levine JD, Messing RO. Nociceptor sensitization by extracellular signal-regulated kinases. J Neurosci 2001;21:6933-6939

[3] Altier N, Stewart J. Tachykinin NK-1 and NK-3 selective agonists induce analgesia in the formalin test for tonic pain following intra-VTA or intra-accumbens microinfusions. Behav Brain Res 1997;89:151-165.

[4] Altier N, Stewart J.The role of dopamine in the nucleus accumbens in analgesia. Life Sci 1999;65:2269-2287.

[5] Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 1984;7:309-338.

[6] Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. Prog Neurobiol 1995;46:575-605.

[7] Burgess SE, Gardell LR, Ossipov MH, Malan TP, Jr., Vanderah TW, Lai J, Porreca F. Time-dependent descending facilitation from the rostral ventromedial medulla maintains, but does not initiate, neuropathic pain. J Neurosci 2002;22:5129-5136.

[8] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;3:55-63.

[9] Dubner R. The neurobiology of persistent pain and its clinical implications. Suppl Clin Neurophysiol 2004;57:3-7.

[10] Dubner R, Ren K. Brainstem mechanisms of persistent pain following injury. J Orofac Pain 2004;18:299-305.

[11] Dujardin E, Jurgens U. Afferents of vocalization-controlling periaqueductal regions in the squirrel monkey. Brain Res 2005;1034:114-131.

[12] Fardin V, Oliveras JL, Besson JM. A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat. I. The production of behavioral side effects together with analgesia. Brain Res 1984;306(1-2):105-23.

[13] Ferreira SH. Prostaglandin, aspirin-like drugs and analgesia. Nat.New Biol 1972;240:200–203

[14] Ferreira SH, Lorenzetti BB, De Campos DI. Induction, blockade and restoration of a persistent hypersensitive state. Pain 1990;42:365-371.

[15] Ferreira SH, Nakamura M, Abreu Castro MS. The hyperalgesic effects of prostacyclin and prostaglandin E2. Prostaglandins 1978;16:31–37.

[16] Fries P, Neuenschwander S, Engel AK, Goebel R, Singer W. Rapid feature selective neuronal synchronization through correlated latency shifting. Nat Neurosci. 2001 Feb;4(2):194-200.

[17] Gardell LR, Vanderah TW, Gardell SE, Wang R, Ossipov MH, Lai J, Porreca F. Enhanced evoked excitatory transmitter release in experimental neuropathy requires descending facilitation. J Neurosci 2003;23:8370-8379.

[18] Gear RW, Aley KO, Levine JD. Pain-induced analgesia mediated by mesolimbic reward circuits. J Neurosci 1999;19:7175-7181.

[19] Gear RW, Levine JD. Antinociception produced by an ascending spino-supraspinal pathway. J Neurosci 1995;15:3154-3161.

[20] Gebhart GF, Sandkuhler J, Thalhammer JG, Zimmermann M. Quantitative comparison of inhibition in spinal cord of nociceptive information by stimulation in

periaqueductal gray or nucleus raphe magnus of the cat. J Neurophysiol 1983;50:1433-1445.

[21] Gu XL, Yu LC. Involvement of opioid receptors in oxytocin-induced antinociception in the nucleus accumbens of rats. J Pain 2007;8:85-90.

[22] Guo W, Robbins MT, Wei F, Zou S, Dubner R, Ren K. Supraspinal brain-derived neurotrophic factor signaling: a novel mechanism for descending pain facilitation. J Neurosci 2006;26:126-137.

[23] Hammond DL, Presley R, Gogas KR, Basbaum AI. Morphine or U-50,488 suppresses Fos protein-like immunoreactivity in the spinal cord and nucleus tractus solitarii evoked by a noxious visceral stimulus in the rat. J Comp Neurol 1992;315:244-253.

[24] Han JS, Xuan YT. A mesolimbic neuronal loop of analgesia: I. Activation by morphine of a serotonergic pathway from periaqueductal gray to nucleus accumbens. Int J Neurosci 1986;29:109-117.

[25] Henriksson KG. Hypersensitivity in muscle pain syndromes. Curr Pain Headache Rep 2003;7:426-432.

[26] Honore P, Buritova J, Besson JM. Carrageenin-evoked c-Fos expression in rat lumbar spinal cord: the effects of indomethacin. Eur J Pharmacol 1995;272:249-259.

[27] Jang JH, Kim DW, Sang Nam T, Se Paik K, Leem JW. Peripheral glutamate receptors contribute to mechanical hyperalgesia in a neuropathic pain model of the rat. Neuroscience. 2004;128(1):169-76.

[28] Jensen TS, Yaksh TL. Brainstem excitatory amino acid receptors in nociception: microinjection mapping and pharmacological characterization of glutamate-sensitive sites in the brainstem associated with algogenic behavior.Neuroscience. 1992;46(3):535-47.

[29] Knyihar E, Csillik B. Plasticity of nociception: recent advances in function-oriented structural pain research. Ideggyogy Sz 2006;59:87-97.

[30] Kovelowski CJ, Ossipov MH, Sun H, Lai J, Malan TP, Porreca F Supraspinal cholecystokinin may drive tonic descending facilitation mechanisms to maintain neuropathic pain in the rat. Pain 2000;87:265-273.

[31] Kretz R. Local cobalt injection: a method to discriminate presynaptic axonal from postsynaptic neuronal activity. J Neurosci Methods1984;11:129-135.

[32] Krnjević K, Puil E. Halothane suppresses slow inward currents in hippocampal slices. Can J Physiol Pharmacol 1988;66(12):1570-5.

[33] Ma QP, Han JS. Neurochemical studies on the mesolimbic circuitry of antinociception. Brain Res 1991;566(1-2):95-102.

[34] Ma QP, Shi YS, Han JS. Further studies on interactions between periaqueductal gray, nucleus accumbens and habenula in antinociception. Brain Res 1992;583:292-295.

[35] Magdalena CM, Navarro VP, Park DM, Stuani MB, Rocha MJ. C-fos expression in rat brain nuclei following incisor tooth movement. J Dent Res 2004; 83:50-54.

[36] Magnusson JE, Martin RV. Additional evidence for the involvement of the basal ganglia in formalin-induced nociception: the role of the nucleus accumbens. Brain Res 2002;942:128-132.

[37] Martin HA, Basbaum AI, Kwiat GC, Goetzl EJ, Levine JD. Leukotriene and prostaglandin sensitization of cutaneous high-threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. Neuroscience 1987;22:651-659.

[38] McMullan S, Lumb BM. Midbrain control of spinal nociception discriminates between responses evoked by myelinated and unmyelinated heat nociceptors in the rat. Pain 2006a;124:59-68.

[39] McMullan S, Lumb BM. Spinal dorsal horn neuronal responses to myelinated versus unmyelinated heat nociceptors and their modulation by activation of the periaqueductal grey in the rat. J Physiol 2006b;576:547-556.

[40] Miki K, Fukuoka T, Tokunaga A, Kondo E, Dai Y, Noguchi K. Differential effect of brain-derived neurotrophic factor on high-threshold mechanosensitivity in a rat neuropathic pain model. Neurosci Lett 2000;278(1-2):85-8.

[41] Millan MJ The induction of pain: an integrative review. Prog Neurobiol 1999;57:1-164.

[42] Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355-474.

[43] Millan MJ, Audinot V, Honore P, Bervoets K, Veiga S, Brocco M. Blockade of NMDA receptors in the nucleus accumbens elicits spontaneous tail-flicks in rats.Eur J Pharmacol 2000;388:37-47.

[44] Mitsui T, Kakizaki H, Matsuura S, Tanaka H, Yoshioka M, Koyanagi T. Chemical bladder irritation provokes c-fos expression in the midbrain periaqueductal gray matter of the rat. Brain Res 2003;967:81-88.

[45] Monhemius R, Green DL, Roberts MH, Azami J. Periaqueductal grey mediated inhibition of responses to noxious stimulation is dynamically activated in a rat model of neuropathic pain. Neurosci Lett 2001;298:70-74.

[46] Neto FL, Schadrack J, Ableitner A, Castro-Lopes JM, Bartenstein P, Zieglgansberger W, Tolle TR. Supraspinal metabolic activity changes in the rat during adjuvant monoarthritis. Neuroscience 1999;94:607-621.

[47] Oliveira MA, Prado WA. Role of PAG in the antinociception evoked from the medial or central amygdala in rats. Brain Res Bull 2001;54:55-63.

[48] Ossipov MH, Bian D, Malan TP Jr, Lai J, Porreca F. Lack of involvement of capsaicin-sensitive primary afferents in nerve-ligation injury induced tactile allodynia in rats. Pain 1999;79(2-3):127-33.

[49] Pace MC, Mazzariello L, Passavanti MB, Sansone P, Barbarisi M, Aurilio C. Neurobiology of pain. J Cell Physiol 2006;209:8-12.

[50] Parada CA, Vivancos GG, Tambeli CH, de Queiroz Cunha F, Ferreira SH. Activation of presynaptic NMDA receptors coupled to NaV1.8-resistant sodium channel C-fibers causes retrograde mechanical nociceptor sensitization. Proc Natl Acad Sci U S A 2003;100:2923-2928.

[51] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1986.

[52] Pelegrini-da-Silva A, Martins AR, Prado WA. A new role for the renin-angiotensin system in the rat periaqueductal gray matter: angiotensin receptor-mediated modulation of nociception. Neuroscience 2005;132:453-463.

[53] Pertovaara A. Plasticity in descending pain modulatory systems. Prog Brain Res 2000;129:231-242.

[54] Pertovaara A, Kontinen VK, Kalso EA. Chronic spinal nerve ligation induces changes in response characteristics of nociceptive spinal dorsal horn neurons and in their descending regulation originating in the periaqueductal gray in the rat. Exp Neurol 1997;147:428-436.

[55] Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci 2002;25:319-325.

[56] Prado WA, Roberts MH. An assessment of the antinociceptive and aversive effects of stimulating identified sites in the rat brain. Brain Res1985;340:219-228.

[57] Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 1957;111:409-419.

[58] Randich A, Aicher SA. Medullary substrates mediating antinociception produced by electrical stimulation of the vagus. Brain Res 1988;445:68-76.

[59] Sandkuhler J. The organization and function of endogenous antinociceptive systems. Prog Neurobiol 1996;50:49-81.

[60] Sommer C. Is serotonin hyperalgesic or analgesic? Curr Pain Headache Rep 2006;10:101-106.

[61] Stamford JA. Descending control of pain. Br J Anaesth 1995;75:217-227.

[62] Tambeli CH, Oliveira MC, Clemente JT, Pelegrini-da-Silva A, Parada CA; A novel mechanism involved in 5-hydroxytryptamine-induced nociception: the indirect activation of primary afferents. Neuroscience 2006;141:1517-1524.

[63] Tambeli CH, Parada CA, Levine JD, Gear RW. Inhibition of tonic spinal glutamatergic activity induces antinociception in the rat. Eur J Neurosci 2002;16:1547-1553.

[64] Tambeli CH, Quang P, Levine JD, Gear RW. Contribution of spinal inhibitory receptors in heterosegmental antinociception induced by noxious stimulation. Eur J Neurosci 2003;18:2999-3006.

[65] Taylor BK, Joshi C, Uppal H. Stimulation of dopamine D2 receptors in the nucleus accumbens inhibits inflammatory pain. Brain Res 2003;987:135-143.

[66] Tseng LF, Wang Q. Forebrain sites differentially sensitive to beta-endorphin and morphine for analgesia and release of Met-enkephalin in the pentobarbital-anesthesized rat. J Pharmacol Exp Ther 1992;261(3):1028-36.

[67] Urban MO, Zahn PK, Gebhart GF. Descending facilitatory influences from the rostral medial medulla mediate secondary, but not primary hyperalgesia in the rat. Neuroscience 1999;90:349-352.

[68] Urca G, Raigorodsky G. Behavioral classification of excitatory amino acid receptors in mouse spinal cord. Eur J Pharmacol. 1988;153(2-3):211-20.

[69] Vanegas H, Schaible HG. Descending control of persistent pain: inhibitory or facilitatory? Brain Res Brain Res Rev 2004;46:295-309.

[70] Vera-Portocarrero LP, Zhang ET, Ossipov MH, Xie JY, King T, Lai J, Porreca F. Descending facilitation from the rostral ventromedial medulla maintains nerve injury-induced central sensitization. Neuroscience 2006; 140:1311-1320.

[71] Villarreal CF, Kina VA, Prado WA. Antinociception induced by stimulating the anterior pretectal nucleus in two models of pain in rats. Clin Exp Pharmacol Physiol 2004a;31:608-613.

[72] Villarreal CF, Kina VA, Prado WA. Participation of brainstem nuclei in the pronociceptive effect of lesion or neural block of the anterior pretectal nucleus in a rat model of incisional pain. Neuropharmacology 2004b;47:117-127.

[73] Vivancos GG, Parada CA, Ferreira SH. Opposite nociceptive effects of the arginine/NO/cGMP pathway stimulation in dermal and subcutaneous tissues. Br J Pharmacol 2003;138:1351-1357.

[74] Wagner R, Deleo JA. Pre-emptive dynorphin and N-methyl-D-aspartate glutamate receptor antagonism alters spinal immunocytochemistry but not allodynia following complete peripheral nerve injury. Neuroscience 1996;72(2):527-34.

[75] Wei F, Dubner R, Ren K. Nucleus reticularis gigantocellularis and nucleus raphe magnus in the brain stem exert opposite effects on behavioral hyperalgesia and spinal Fos protein expression after peripheral inflammation. Pain 1999;80:127-141.

[76] Willis WD, Westlund KN (Neuroanatomy of the pain system and of the pathways that modulate pain. J Clin Neurophysiol 1997;14:2-31.

[77] Xiong W, Yu LC. Involvements of mu- and kappa-opioid receptors in morphine-induced antinociception in the nucleus accumbens of rats. Neurosci Lett 2006;399:167-170.

[78] Xuan YT, Shi YS, Zhou ZF, Han JS. Studies on the mesolimbic loop of antinociception--II. A serotonin-enkephalin interaction in the nucleus accumbens. Neuroscience 1986;19:403-409.

[79] Yu LC, Han JS. Involvement of arcuate nucleus of hypothalamus in the descending pathway from nucleus accumbens to periaqueductal grey subserving an antinociceptive effect. Int J Neurosci 1989;48:71-78.

[80] Zhang DX, Owens CM, Willis WD. Two forms of inhibition of spinothalamic tract neurons produced by stimulation of the periaqueductal gray and the cerebral cortex. J Neurophysiol 1991;65:1567-1579.

[81] Zhang L, Zhang Y, Zhao ZQ. Anterior cingulate cortex contributes to the descending facilitatory modulation of pain via dorsal reticular nucleus. Eur J Neurosci 2005;22:1141-1148.

[82] Zhuo M. Targeting central plasticity: a new direction of finding painkillers. Curr Pharm Des 2005;11:2797-2807.

[83] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109-10.

CONCLUSÃO

Este estudo demonstrou que o Núcleo Accumbens (Nacc) e a Substância Cinzenta Periaquedutal (PAG) modulam de modo distinto a dor inflamatória aguda e crônica.

A PAG teve sua influência inibitória mais uma vez confirmada na hiperalgesia aguda, porém não mostrou efeito algum na modulação da hiperalgesia persistente.

O Nacc exerce uma influência inibitória na hiperalgesia aguda, contribuindo para a redução de sua intensidade. Em contraste, uma influência facilitatória do Nacc contribui para a manutenção da hiperalgesia persistente.

Foi também observado que, pela estimulação da atividade do Nacc, foi possível restaurar a magnitude da hiperalgesia que havia sido reduzida por um analgésico periférico (Dipirona).

Estes resultados trazem um conceito inovador na modulação nociceptiva descendente endógena, indicando um possível mecanismo fisiopatológico que poderia contribuir para a manutenção da hiperalgesia persistente e também no processo de reinstalação da mesma após um período quiescente, o que poderia estar relacionado à recorrência de episódios de dor em processos crônicos. A modulação nociceptiva descendente endógena parece, portanto, exercer uma influência inibitória ou facilitatória dependendo da duração do processo patológico.

REFERÊNCIAS (*)

Aley KO, Messing RO, Mochly-Rosen D, Levine JD. Chronic hypersensitivity for inflammatory nociceptor sensitization mediated by the epsilon isozyme of protein kinase C. J Neurosci. 2000 Jun 15;20(12):4680-5.

Reynolds DV. Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science.1969 Apr 25;164(878):444-5

Sachs D, Cunha FQ, Poole S, Ferreira SH. Tumour necrosis factor-alpha, interleukin-1beta and interleukin-8 induce persistent mechanical nociceptor hypersensitivity. Pain 96 (2002) 89–97.

(*) De acordo com a norma UNICAMP/FOP, baseada no International Committe of Medical Journal Editors – Grupo de Vancouver. Abreviaturas dos periódicos em conformidade com o Medline.

ANEXO

UNICAMP

Universidade Estadual de Campinas
Instituto de Biologia

CEEA-IB-UNICAMP

Comissão de Ética na Experimentação Animal
CEEA-IB-UNICAMP

C E R T I F I C A D O

Certificamos que o Protocolo nº **1049-1**, sobre **"PARTICIPAÇÃO DA VIA DESCENDENTE INIBITÓRIA DA NA MODULAÇÃO HIPERNOCICEPÇÃO PERSISTENTE EM RATOS"**, sob a responsabilidade de **Profa. Dra. Adriana Pelegrine da Silva / Priscila Tiemi Kawashita**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal (CEEA)-IB-UNICAMP em reunião de 26 de junho de 2006.

C E R T I F I C A T E

We certify that the protocol nº **1049-1**, entitled **"INVOLVEMENT OF INHIBITORY DESCENDING PATHWAY IN THE MODULATION OF TONIC HYPERALGESIA IN RATS"**, is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - UNICAMP) on June 26, 2006.

Campinas, 21 de agosto de 2008.

2ª. VIA

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

CEEA/IB – Unicamp
Caixa Postal 6109
13083-970 Campinas, SP – Brasil

Telefone: (19) 3788-6359
Telefax: (19) 3788-6356
E-mail: ceea@cemib.unicamp.br
http://www.ib.unicamp.br/institucional/ceea/index.htm