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Effect of a metabotropic glutamate receptor 5 antagonist, MPEP, on the nociceptive response induced by intrathecal injection of excitatory aminoacids, substance P, bradykinin or cytokines in mice

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ABSTRACT

Metabotropic glutamate receptors (mGluRs) are expressed abundantly in the spinal cord and have been shown to play important roles in the modulation of nociceptive transmission and plasticity. In this study, the involvement of metabotropic glutamate receptor 5 ($mGluR_5$) in the nociceptive response induced by intrathecal injection (i.t.) of excitatory aminoacids, substance P (SP), bradykinin (BK) and cytokines in mice was demonstrated. The administration of 2-methyl-6-(phenylethynyl)-pyridine (MPEP; 10–50 nmol/site, i.t.) caused a significant inhibition in the nociceptive response induced by glutamate and *trans*-ACPD with maximal inhibitory effects of 36±7% and 56±5%, respectively. MPEP completely failed to affect the nociception induced by α-amino-3-hydroxy-5-mehtyl-4-isoxazolepropionic acid (AMPA; 135 pmol/site), kainate (110 pmol/site) and *N*-methyl-D-aspartate (NMDA; 450 pmol/site). MPEP also reduced the nociceptive response induced by SP (135 ng/site, i.t.), BK (0.1 μg/site), tumor necrosis factor-alpha (TNF-α; 0.1 pg/site) and interleukin-1beta (IL-1β; 1 pg/site) with maximal inhibitions of 29±5%, 37±5%, 83±3% and 88±1%, respectively. Together, these results indicate the involvement of mGluRs, more specifically of subtype-5, in the nociceptive response induced by i.t. injection of excitatory aminoacids, SP, BK and cytokines in mice.

1. Introduction

Nociceptors are specialized sensory neurons that detect chemical, physical or mechanical stimuli that could cause tissue damage. Several agents enhance the excitability of the nociceptor, including neurotransmitters and neuromodulators as substance P (SP), glutamate, bradykinin (BK), prostaglandin, histamine, serotonin, cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-18 among others (Griffis et al., 2006). These substances bind to receptors and activate signaling pathways, among these are protein kinases A and C, calcium/calmodulin-dependent protein kinase, and mitogen-activated protein kinases (MAPKs) (Bevan 1999; Ji and Stricharstz, 2004). Thus, nociceptors not only signal acute pain but also contribute to persistent and pathological pain conditions (allodynia) that occur in the setting of injury, wherein pain is produced by innocuous stimuli (Burgess et al., 1989; Julius and Basbaum, 2001).

Preclinical evidence suggests that the spinal cord dorsal horn glutamatergic system has a crucial role in tissue injury-induced pain (Mao et al., 1992; Petralia et al., 1992; Furuyama et al., 1993; Kolhekar et al., 1993). Glutamate activates ionotropic glutamate receptors (iGluRs) which include NMDA, AMPA and kainate receptors, and the metabotropic glutamate receptors (mGluRs) which are coupled to G proteins. To date, eight mGluR subtypes have been cloned and are termed mGluRs 1–8. These are broadly classified into I (mGluRs 1 and 5), II (mGluRs 2 and 3) and III (mGluRs 4, 6, 7 and 8) groups, based on their sequence homologies, pharmacology and coupling to intracellular effector systems (Conn and Pin, 1997). The $mGluR_1$ and $mGluR_5$ have been localized in small and medium diameter dorsal root ganglion neurons, in primary afferent axons and in spinal cord dorsal horn neurons (Hudson et al., 2002; Walker et al., 2001b). Moreover, $mGluR_1$ and $mGluR_5$ have been predominantly implicated in nociceptive transmission and central sensitization (Crawford et al., 1999). The involvement of this class of receptors has been demonstrated mainly by the use of selective antagonists that inhibited the nociceptive response induced by different noxious stimuli and by the use of mice deficient in some of these receptors (Walker et al., 2001a,b).

The intrathecal administration of the selective $mGlu_5$ receptor antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999) produced acute antinociceptive effects in the second phase of the formalin model (Karim et al., 2001) and reduced mechanical but not cold hypersensitivity associated with chronic constriction injury

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(CCI) in rats (Fisher et al., 2002). The spinal administration of anti-$mGluR_5$ IgG antibodies reduced cold hyperalgesia in rats with neuropathic pain (chronic pain) (Fundytus et al., 1998). Several authors have also reported that the administration of MPEP attenuates the nociceptive behavior in neuropathic pain models (Gasparini et al., 1999; Walker et al., 2001a,b; Fisher et al., 2002). Finally, electrophysiological recordings in a hemisected spinal cord in an in vitro preparation suggest that $mGluR_5$ receptors may be involved in brief and prolonged spinal nociception (Bordi and Ugolini, 2000).

It is known that B_1 as well as B_2 receptors are constitutively expressed on dorsal root ganglia (DRG) sensory neurones and the superficial laminae of the spinal cord in naive animals (Wotherspoon and Winter, 2000; Ma and Heavens, 2001). The reduction in the behavioral manifestations of activity-dependent central sensitization in the spinal cord, demonstrated by antagonizing the B_2 receptor and in B_2 null mutants, reinforces the conclusion that bradykinin is a centrally acting synaptic modulator that has a particular role in producing acute injury-induced pain hypersensitivity (Wang et al., 2005). Moreover, the B_1 antagonists, des-Arg^{10}HOE140 and des-Arg^9Leu^8-BK (Jones et al., 1999), caused a pronounced reversal of inflammatory hyperalgesia when injected by intrathecal route.

The cytokines, IL-1β and TNF-α, are over expressed in the spinal cord on peripheral inflammatory and neuropathic experimental models of pain (Ji and Stricharstz, 2004; Sung et al., 2005). In addition, IL-1β and TNF-α cause sensitization of peripheral nociceptors (hypernociception) and nociception when administrated intrathecally (Choi et al., 2003; Cunha et al., 2005). Pineau and Lacroix (2007) have reported that either IL-1β or TNF-α is rapidly and transiently expressed in the injured mouse spinal cord.

Therefore, in the present study, we sought to examine the involvement of $mGluR_5$, by using MPEP (a selective $mGlu_5$ receptor antagonist), in the nociceptive response induced by intrathecal injection of excitatory aminoacids, substance P, bradykinin and cytokines in mice.

2. Materials and methods

2.1. Animals

The behavioral experiments were conducted using male Swiss mice (25–35 g) (n=6–8 animals) maintained at 23±2 °C with free access to water and food, under a 12:12 h light/dark cycle. Mice were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resource, the Federal University of Santa Maria, Brazil and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of drug treatments.

2.2. Intrathecal (i.t.) injection

Intrathecal injections were given to awake animals using the method described by Hylden and Wilcox (1980). Briefly, the animals were restrained manually and a 30-gauge needle, attached to a 25 µl microsyringe, was inserted through the skin between the vertebrae into the subdural space of the L5–L6 spinal segments. Injections were given over a period of 5 s.

2.3. Drugs

The following substances were used: L-glutamic acid hydrochloride (glutamate), kainic acid (kainate), (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartic acid (NMDA), capsaicin, cytokines (tumor necrosis factor-alpha and interleukin-1beta), bradykinin (BK), substance P (SP) and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Sigma, St. Louis, USA). All other drugs used were dissolved in a saline solution (0.9% of NaCl, w/v). Control animals received 0.9% saline solution only.

2.4. Nociception induced by algogen in mice

To verify the effect caused by MPEP on the nociceptive response induced by i.t. injection of excitatory aminoacids (EAAs), SP, BK or cytokines, mice received MPEP by intrathecal route (i.t.) (dose range: 1–50 nmol/site) 15 min before i.t. injection of 5 µl of the EAAs, substance P, bradykinin or pro-inflammatory cytokines or vehicle solution. The nociceptive response was elicited by glutamate (an excitatory aminoacid, 175 nmol/site, it.), NMDA (a selective agonist of NMDA-subtype of ionotropic glutamate receptors, 450 pmol/site, i.t.) (Urca and Raigorodsky, 1988), AMPA (a selective agonist of AMPA-subtype of ionotropic glutamate receptors, 135 pmol/site, i.t.) (Brambilla et al., 1998), kainate (a selective agonist of kainate-subtype of ionotropic glutamate receptors, 110 pmol/site, i.t.), *trans*-ACPD (a metabotropic glutamate receptors agonist, 50 nmol/site, i.t.) (Boxall et al., 1998), SP (a NK_1 receptor-selective agonist, 135 ng/site) (Sakurada et al., 1990); IL-1β (1 pg/site) and TNF-α (0.1 pg/site) (Choi et al., 2003) or BK (0.1 µg/site) (Kamei et al., 1999) with minor modifications. A group of mice only received vehicle (0.9% saline solution) by i.t. route. The amount of time the animal spent biting was evaluated following local post-injections of each agonist: glutamate (3 min); AMPA (1 min);

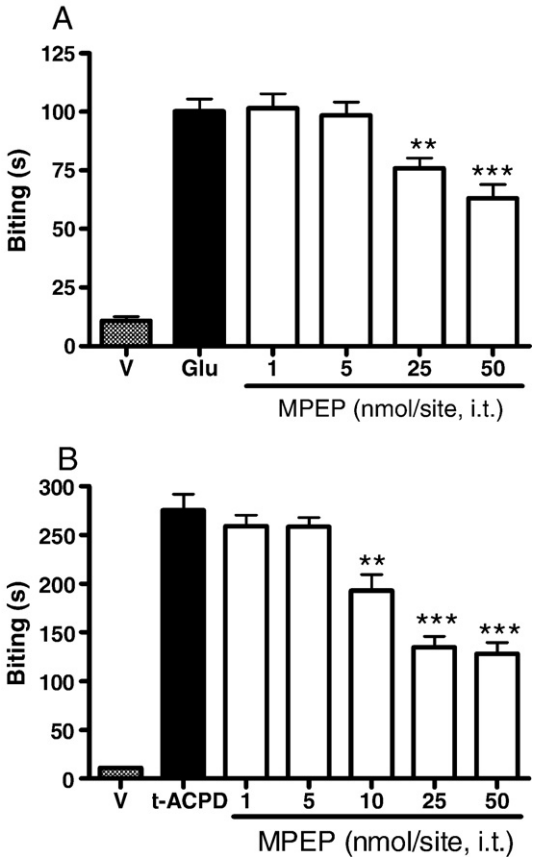

Fig. 1. Effect of i.t. administration of MPEP (1–50 nmol) on glutamate (175 nmol/site, i.t., A) or *trans*-ACPD (50 nmol/site, i.t., B) -induced nociceptive response. Each column represents the mean±S.E.M. for six to eight animals. Asterisks denote the significance levels $^{**}P<0.01$ and $^{***}P<0.001$ compared to the control group values (closed columns) (one-way ANOVA followed by Newman–Keuls test).

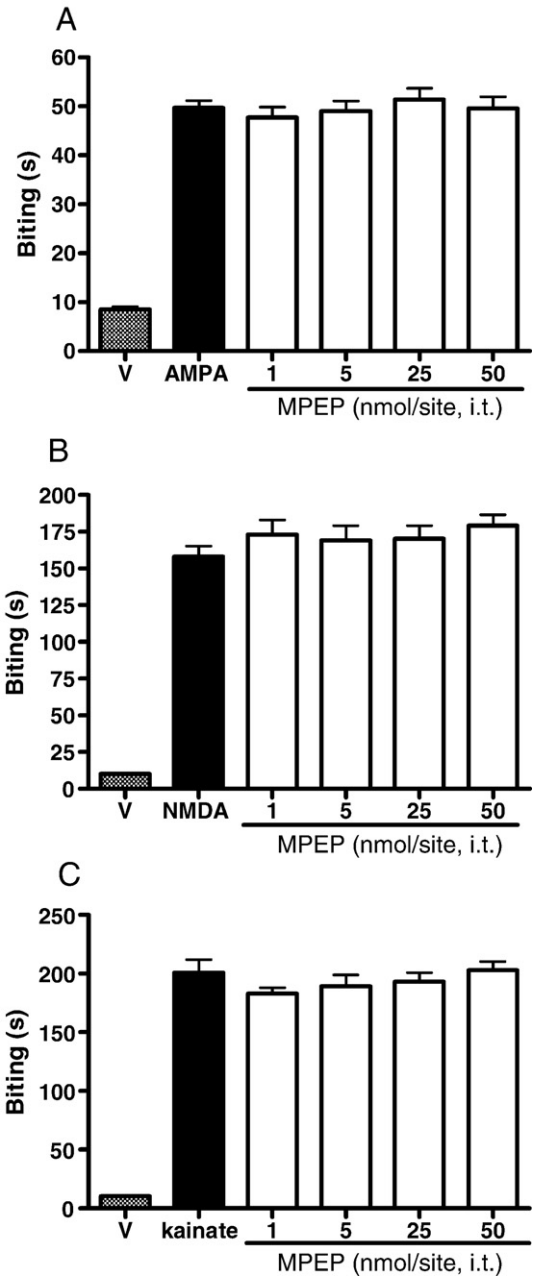

Fig. 2. Effect of i.t. administration of MPEP (1–50 nmol/site, i.t.) on AMPA (135 pmol/site, i.t., A), NMDA (450 pmol/site, i.t., B) or kainate (110 pmol/site, i.t., C) -induced nociceptive response. Each column represents the mean ± S.E.M. for six to eight animals.

NMDA and SP (6 min); *trans*-ACPD, IL-1β and TNF-α (15 min) and BK (30 min). A bite was defined as a single head movement directed at the flanks or hind limbs, resulting in contact of the animal's snout with the target organ.

2.5. Statistical analysis

The results are presented as mean ± S.E.M., except the ID_{50} values (i.e., the dose of MPEP necessary to reduce the nociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} value was determined by linear regression from individual experiments using linear regression GraphPad software (GraphPad software, San Diego, CA, USA). Comparisons between experimental and control groups were performed by ANOVA followed by Newman–Keuls test when appropriated. P values less than 0.05 ($P<0.05$) were considered as indicative of significance.

3. Results

The results presented in Fig. 1(A–B) show that MPEP (10–50 nmol/site, i.t.) inhibited nociceptive response induced by spinal injections of glutamate and *trans*-ACPD in mice. The maximal inhibitory effects of MPEP for nociceptive response induced by glutamate and *trans*-ACPD were 36 ± 7% and 56 ± 5%, respectively.

The calculated mean ID_{50} value (and its respective 95% confidence limits) for the antinociceptive effect caused by MPEP against *trans*-ACPD-induced biting was 36.90 (30.63–44.64) nmol/site. In contrast, MPEP had no significant effect against AMPA, NMDA and kainate mediated nociceptive response in mice (Fig. 2A–C).

The results depicted in Fig. 3(A–B) show that treatment with MPEP (5–50 nmol/site, i.t.) caused a significant inhibition in the nociceptive response induced by i.t. injection of TNF-α and IL-1β when compared to the control group. The maximal inhibitory effects of MPEP for nociceptive response induced by TNF-α and IL-1β were 83 ± 3% and 88 ± 1%, respectively. The calculated mean ID_{50} values (and their respective 95% confidence limits) for the antinociceptive effect caused by MPEP against TNF-α and IL-1β mediated biting response in mice were 12.76 (10.33–15.75) and 13.17 (10.26–16.89) nmol/site, respectively.

Fig. 4(A) shows that MPEP (5–50 nmol/site, i.t.) significantly inhibited the nociceptive response induced by the i.t. injection of SP with maximal inhibition of 29 ± 5%. MPEP (25–50 nmol/site, i.t.)

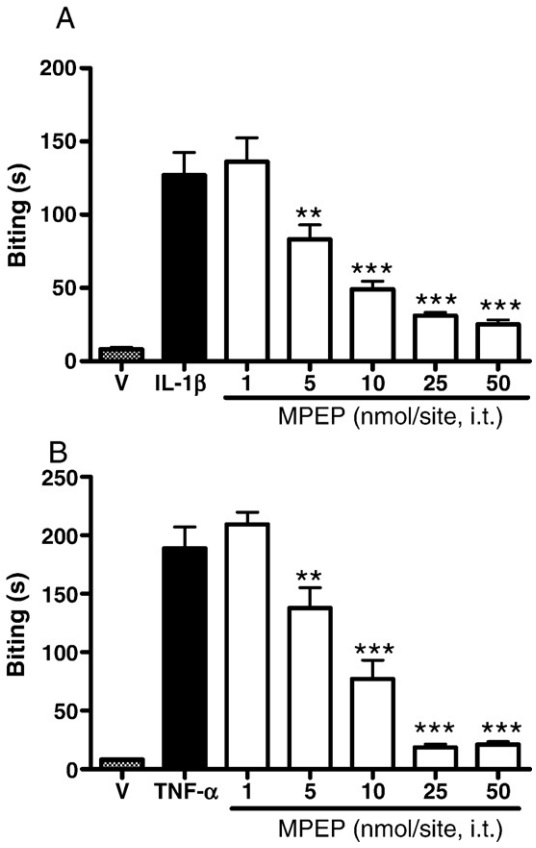

Fig. 3. Effect of i.t. administration of MPEP (1–50 nmol/site, i.t.) on IL-1β (1 pg/site, i.t., A) or TNF-α (0.1 pg/site, i.t., B) -induced nociceptive response. Each column represents the mean ± S.E.M. for six to eight animals. Asterisks denote the significance levels $^{**}P<0.01$ and $^{***}P<0.001$ compared to the control group values (closed columns) (one-way ANOVA followed by Newman–Keuls test).

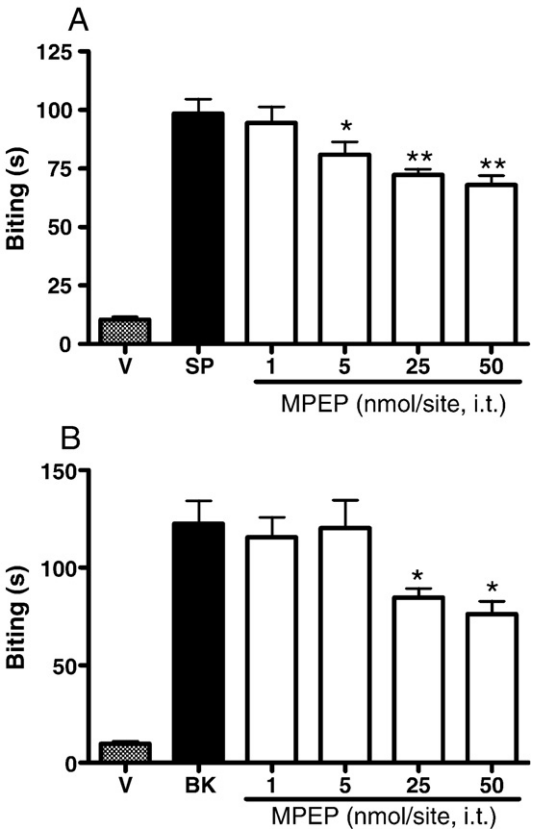

Fig. 4. Effect of i.t. administration of MPEP (1–50 nmol/site, i.t.) on substance P (SP, 135 ng/site, i.t., A) or BK (0.1 μg/site, i.t., B) -induced nociceptive response. Each column represents the mean±S.E.M. for six to eight animals. Asterisks denote the significance levels *$P<0.05$ and **$P<0.01$ compared to the control group values (closed columns) (one-way ANOVA followed by Newman–Keuls test).

significantly inhibited the nociceptive response caused by the injection of BK with a maximal inhibitory effect of 37±5% (Fig. 4B).

4. Discussion

MPEP is a highly potent and selective $mGluR_5$ antagonist that exhibits low nanomolar affinity for $mGluR_5$ (Gasparini et al., 1999). In the present study, administration of MPEP attenuated the nociceptive response caused by glutamate (Glu) and *trans*-ACPD but did not modify the response caused by i.t. injection of AMPA, NMDA and kainate. Based on these findings, we suggest an interaction of MPEP with metabotropic receptors, thus reducing the interaction of *trans*-ACPD with such receptors. It is also possible that part of the effect of the agonist *trans*-ACPD might be due to an activation of other metabotropic glutamate receptors subtypes. Data from the literature indicate that *trans*-ACPD could activate both group I and group II metabotropic glutamate receptors (Bond and Lodge, 1995; Dolan and Nolan, 2000). This information could help explain why MPEP, a selective subtype I ($mGlu_5R$) antagonist, was capable to only reduce 56% of the nociception caused by *trans*-ACPD. Therefore, these results demonstrated the involvement of $mGluR_5$ in the nociceptive response induced by i.t. injection of excitatory aminoacids.

It has been demonstrated that the intrathecal administration of the selective $mGluR_5$ antagonist, MPEP, attenuated (*R*, *S*)-3,5-dihydroxyphenylglycine (DHPG) (a $mGluR_1$ and $mGluR_5$ selective agonist)-induced nociceptive behavior and reduced the second phase of the formalin test in a dose-dependent manner, suggesting that this receptor contributes to the second phase of nociceptive behavior (Karim et al., 2001). Moreover, this antagonist attenuated the first phase of the formalin test at higher doses, suggesting that $mGluR_5$ is also involved in acute nociceptive transmission (Karim et al., 2001; Gabra et al., 2008). This is consistent with a previous study showing a reduction of acute nociceptive transmission by administration of MPEP and by group I mGluR antisense knockdown (Young et al., 1998). Additionally, the DHPG-evoked sensitivity to cold in rats was prevented by i.t. pretreatment with the $mGlu_5$ receptor antagonist, MPEP, suggesting that acute activation of spinal $mGlu_5$ receptors is involved in the induction of cold hypersensitivity. On the other hand, intrathecal injection of MPEP failed to attenuate cold hypersensitivity evoked by either i.t. DHPG or chronic constriction injury (CCI). Another study (Fisher et al., 2002) confirms that spinal administration of MPEP only reduced mechanical but not cold hypersensitivity associated with CCI in rats.

Also relevant are the findings showing that MPEP given by i.t. route produced an inhibition of the SP induced biting response in mice. In particular, SP may mediate signaling at the synapse between primary nociceptive afferent fibers and spinal dorsal horn neurons, which are functionally important for the nociception. As pointed out in the Introduction section of this study, besides the hyperalgesic response caused by SP, this neuropeptide also co-exists with glutamate in sensory fibers (Hökfelt, 1991; Levine et al., 1993; Otsuka and Yoshioka, 1993). In addition, the modulatory control of mGlu receptors on SP release occurs in the rat trigeminal nucleus slices (Cuesta et al., 1999) as well as in other sensory structures such as the spinal cord (Davies and Watkins, 1982), thalamus (Salt and Eaton, 1995) and hippocampus (Koerner and Cotman, 1981). There is convincing experimental evidence showing that i.t. injection of SP results in a nociceptive response, an effect that is mediated by its interaction with NK_1 receptors (Malmberg and Yaksh, 1992). The selective tachykinin NK_1 receptor antagonists have been shown to be active in the second phase of the formalin test, used as a model of inflammatory pain (Chapman and Dickenson, 1993; Rupniak et al., 1996). Moreover, Yaskh et al. (1999) have shown that spinal neurokinin 1, NMDA, and non-NMDA receptors enhance spinal prostaglandin E_2 release and spinal prostaglandins facilitate release of spinal aminoacids and peptides. Since SP and excitatory aminoacids induced spinal release of glutamate, we can propose that the interaction of glutamate with metabotropic receptors would be the event inhibited by MPEP.

Another interesting outcome of the present study is the demonstration that MPEP inhibited BK-induced nociceptive behavior in mice. BK and its related kinins are vasoactive peptides which have an important role as inflammatory mediators and are normally released following tissue trauma or infection. Once released, BK can release most inflammatory and algogenic substances, namely products derived from arachidonic acid pathways, cytokines and nitric oxide (Calixto et al., 2000, 2001). Indeed, BK increases glutamate release from astrocytes and calcium levels in neurons (Parpura et al., 1994). In agreement, Wang et al. (2005) have reported that BK is released in the spinal cord in response to nociceptor inputs and acts as a synaptic neuromodulator, potentiating glutamatergic synaptic transmission to produce pain hypersensitivity. Thus, BK is a modulator of glutamatergic synaptic transmission in the spinal cord with presynaptic and postsynaptic actions (Wang et al., 2005). Therefore, based on the considerations above that BK potentiates glutamatergic synaptic transmission, we can suggest that BK injected by i.t. route, induced spinal release of glutamate that interacts with metabotropic receptors to induce the nociceptive response. The interaction of glutamate with these receptors would be the event inhibited by the antagonist of metabotropic receptor, MPEP. Moreover, results obtained using i.t. injection of kinin receptor antagonists further support the concept that endogenous B_1 or B_2 receptor agonists are produced in the spinal cord and their inhibition produces analgesia in models of acute, subchronic and chronic nociception (Calixto et al., 2000, 2001; Couture et al., 2001; Ferreira et al., 2002). These findings strongly support the proposal that kinins acting at receptors

in the spinal cord seem to control the process of pain transmission in vivo.

The present study also demonstrated that MPEP administered by i.t. route produces an inhibition of nociceptive response caused by i.t. injection of cytokines, IL-1β and TNF-α. Cytokines, such as TNF-α, IL-1α, IL-1β and IFN-γ, are capable of inducing nociceptive behavior when injected by i.t route, in the absence of peripheral nociceptive stimulation (Tadano et al., 1999; Choi et al., 2003). Narita et al. (2008) have demonstrated that the IL-1β and TNF-α released within the spinal cord play a critical role in the development of chronic inflammatory pain induced by the intraplantar injection of Freund's adjuvant (CFA). This may be related to the induction of glutamate and SP release from nerve terminals, through direct activation of these by pro-inflammatory cytokines (Tadano et al., 1999). Based on the considerations above and our data, we can infer that cytokines, injected by i.t. route, induce spinal release of glutamate that interacts with metabotropic receptors to induce the nociceptive response and the interaction of glutamate with these receptors would be the event inhibited by the antagonist of metabotropic receptors, MPEP. In addition, another findings demonstrated that peripheral (Fundytus et al., 1998) or intracisternal administration (Hama, 2003) of MPEP blocked IL-1β-induced mechanical allodynia and mirror-image mechanical allodynia produced by a subcutaneous injection of 10 pg of IL-1β in the orofacial area in rats (Ferreira et al., 1988; Vila et al., 2005). The results presented here revealed that MPEP, administered by i.t. route, elicits an inhibition of the TNF-α-induced nociception. Cumiskey et al. (2007) have reported that the impairment of early-LTP (long-term potentiation) by TNF-α is significantly attenuated by prior application of the selective $mGluR_5$ antagonist. Additionally, it suggests that metabotropic glutamate receptor activation, involving $mGlu_5$, causes p38 activation, which leads to the TNF-α inhibition (Bolshakov et al., 2000; Butler et al., 2004).

Taken together, the results of the present study show the involvement of metabotropic glutamate receptors, more specifically of subtype-5, in the nociceptive response induced by i.t. injection of excitatory aminoacids, SP, BK and cytokines in mice.

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