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Metabotropic glutamate receptor 5 and dorsal raphe serotonin release in inflammatory pain in rat

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Abstract

In this study, we evaluated the effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective antagonist of metabotropic glutamate subtype 5 receptors ($mGlu_5$), delivered through different paths on dorsal raphe serotonin (5-HT) and on thermoceptive responses in rats with inflammatory pain. Intraplantar formalin and carrageenan increased 5-HT (137 ± 11% and 212 ± 6% of pre-injection baseline, respectively) and reduced nociceptive threshold (23 ± 7% and 19 ± 3% of pre-injection baseline, respectively). MPEP (2 mg/kg i.p.) further enhanced formalin and carrageenan-induced 5-HT increases (180 ± 11% and 260 ± 12% of pre-injection baseline, respectively) and reduced thermal hyperalgesia (71 ± 8% and 80 ± 10% of pre-injection baseline, respectively). MPEP (1 mM) through microdialytic probe into the dorsal raphe did not change formalin- or carrageenan-induced 5-HT increases (147 ± 10% and 189 ± 10% of pre-injection baseline, respectively) and thermal hyperalgesia (35 ± 8% and 25 ± 9% of pre-injection baseline, respectively). Finally, MPEP (30 nmol/rat) into the hind paw reduced the formalin- and carrageenan-induced 5-HT increase (108 ± 3% and 126 ± 7% of pre-injection baseline, respectively) and thermal hyperalgesia (77 ± 6% and 117 ± 7% of pre-injection baseline, respectively). Dorsal raphe serotonergic neurons activity increased following a peripherally induced inflammatory injury. In these conditions, peripheral but not dorsal raphe $mGlu_5$ receptors blockade prevented over activation of dorsal raphe serotonergic neurons and reversed thermal hyperalgesia.

Keywords: $mGlu_5$; MPEP; Serotonin; Dorsal raphe; Microdialysis; Thermal nociception

1. Introduction

Midbrain dorsal raphe is involved in descending pathway that controls noxious inputs to the spinal cord and participates in the normal physiological responses to noxious stimulation (Wang and Nakai, 1994; Watkins et al., 1998; Prado and Foganello, 2000). Inhibition of the nociceptive responses of spinal dorsal horn by dorsal raphe stimulation might be partly achieved by indirect control of the nucleus raphe magnus on nociceptive afferent spinal cord neurons, and partly by direct modulation of the dorsal raphe on spinal nociceptive messages (Wang and Nakai, 1994). Dorsal raphe is the origin of the great majority of serotonergic fibres which innervate structures involved in pain modulation. It is well documented that 5-hydroxytryptamine (serotonin, 5-HT) is released in dorsal horn following sciatic nerves stimulation (Tyce and Yaksh, 1981), carrageenan-induced inflammatory pain (Zhang et al., 2000) and chronic pain states (Schoenen et al., 1985; Godefroy et al., 1987). Pain-induced changes on dorsal raphe 5-HT release still remains unexplored. In addition to 5-HT, supraspinal glutamatergic system evokes antinociception from several brain structures including the dorsal raphe (Behbehani and Fields, 1979; Urca et al., 1980; Jensen and Yaksh, 1984). The excitatory effects of glutamate on neuronal cell bodies and dendrites is mediated either through interaction with ionotropic glutamate receptors or by G protein-coupled metabotropic glutamate (mGlu) receptors (Salt, 1986; Baranauskas and Nistri, 1998). A specific role for group I mGlu receptors ($mGlu_1$ and $mGlu_5$ receptors) in nociceptive processing has been demonstrated by previous studies (Vidnyanszky et al., 1994; Romano et al., 1995;

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Valerio et al., 1997; Boxall et al., 1998; Fisher and Coderre, 1998; Berthele et al., 1999; Jia et al., 1999). Although $mGlu_1$ and $mGlu_5$ are highly related receptors, the development of selective antagonists and, in particular, the identification of the selective and potent $mGlu_5$ receptor antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), has enabled to further define the contribution of $mGlu_5$ receptor in nociceptive processes. The involvement of $mGlu_5$ receptors in modulating pain has been shown peripherally (deGroot et al., 2000; Walker et al., 2001a,b) and centrally both at spinal (Bordi and Ugolini, 2000; Dogrul et al., 2000) and supraspinal (Salt and Binns, 2000; Palazzo et al., 2001, 2002) levels in different models of pain (deGroot et al., 2000; Walker et al., 2001a,b). MPEP has shown to penetrate the blood–brain barrier when systemically administered (Bowes et al., 1999; Gasparini et al., 1999). Thus, peripheral and central $mGlu_5$ blockade may participate in inhibiting nociceptive behaviours, especially in inflammatory pain (Bhave et al., 2001; Walker et al., 2001a,b). There is controversy, however, over the contribution of peripheral or central $mGlu_5$ blockade to analgesia in different chronic pain models such as those associated with nerve injury or persistent inflammation. Moreover, there is no study aimed at investigating the possible $mGlu_5$ receptor-mediated control on dorsal raphe serotonergic pathway in the pathophysiology of inflammatory pain. Due to the key role of serotonergic and glutamatergic neurotransmissions in the descending pathway that inhibits pain, in this study, we examined changes in dorsal raphe 5-HT release and thermoceptive responses in inflammatory pain models with or without $mGlu_5$ receptors blockade. Further insights about neurochemical changes, such as 5-HT release in dorsal raphe, as well as in other areas of pain descending system (periaqueductal grey, rostral ventrolateral medulla, etc.) associated to thermoceptive threshold changes may, in experimental pain conditions, pave the way to further characterize the antinociceptive mechanisms of action of this new potential pain relieving agent.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were housed three per cage under controlled illumination (12-h light/12-h dark cycle; lights on 06:00 h) and standard environmental conditions (ambient temperature 20–22 °C, humidity 55–60%) for at least 1 week before the commencement of experiments. Rat chow and tap water were available ad libitum. All surgery and experimental procedures were done during the light cycle and were approved by the Animal Ethics Committee of The Second University of Naples. Animal care was in compliance with Italian (D.L. 116/92) and EC (O.J. of E.C. L358/1 18/12/86) regulations on the protection of laboratory animals. All efforts were made to reduce both animals number and suffering during the experiments.

2.2. Microdialysis

Microdialysis experiments were performed on freely moving rats. In brief, rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and concentric dialysis probes were implanted with stereotaxic apparatus in the dorsal raphe (7.8 mm posterior to bregma, 7.2 mm ventral to it and 0.2 mm lateral to the midline) using the stereotaxic atlas of Paxinos and Watson (1986). Dialysis probes were constructed as described by Hutson et al. (1985) with 25-G (0.3 mm ID, 0.5 mm OD) stainless steel tubing (A-M systems, Everett, USA). Inlet and outlet cannulae (0.04 mm ID, 0.14 OD) consisted of fused silica tubing (Scientific Glass Engineering, Melbourne, Australia). The microdialysis probes had a 2-mm long tubular dialysis membrane (Enka, Wuppertal, Germany). Animals were allowed to recover from surgery for 20–24 h, then probes were perfused with artificial cerebrospinal fluid (ACSF composition in mM: NaCl, 125; KCl, 2.5; $MgCl_2$, 1.18; and $CaCl_2$, 1.26) at a rate of 0.8 μl/min using a Harvard Apparatus infusion pump (mod. 22). After an initial 60-min equilibration period, dialysate samples were collected every 30 min for a period of time depending of the pain model. Five fractions were collected before inducing pain. Pretreatments with MPEP 2 mg/kg i.p., 1 mM (0.5 μg) through the microdialytic probe or 30 nmol (7 μg) into the rat hind paw were performed after the collection of the fifth dialytic sample. At the end of the experiments, all the rats were deeply anaesthetized with pentobarbital and transcardially perfused with 0.9% NaCl solution followed by 10% formaldehyde solution. The brain was dissected out and fixed in a 10% formaldehyde solution for 48 h. The brain was cut in 40 μm thick slices and observed in a light microscope by the birefringency method (Schenk and Kistler, 1962) to localize the probe tip.

Concentration of 5-HT was determined using high performance liquid chromatography (HPLC) equipment fitted with an electrochemical detector as previously described by Hutson et al. (1985). The composition of the mobile phase was 0.15 mM NaH_2PO_4, 0.01 mM octyl sodium sulphate, 0.5 mM EDTA (pH 3.8 adjusted with phosphoric acid) and 12.5% methanol. Mobile phase was delivered (flow rate: 1 ml/min) by a model 590 pump (Waters Associates, Milforal, USA) into an Ultrasphere 3 μm ODS column (4.6 mm × 7.5 cm; Beckman, San Ramon, USA). The electrochemical detector was an ESA Coulochem mod. 5100A with a dual electrode analytical cell (mod. 5011). The conditioning cell was set at −0.05 V, electrode 1 at +0.10 V and electrode 2 at +0.25 V with respect to palladium reference electrodes. The limit of detection for 5-HT was 2–3 fmol/sample injected with a signal-to-noise ratio of 2. The mean dialysate concentration of 5-HT in the first five samples, before any drug treatment and/or inducing pain, represented the pre-injection baseline ($n = 10$ per group) and data were expressed as percentage of it. In vitro recovery of the microdialysis probe for 5-HT was 23–25%.

2.2.1. Combination of microdialysis and pain states

The same day of dialysates collection groups of animals received either formalin or carrageenan into the paw (see below). These procedures were performed after the collection of the fifth basal sample of perfusate.

Formalin test is a widely used animal model of persistent pain (Dubuisson and Dennis, 1977). Fifty microliters of 5% formaldehyde in 0.9% saline solution was injected subcutaneously into the dorsal surface of the hind paw using a 30-gauge needle.

The carrageenan model of inflammation has been used to examine the effects of drugs on the development of inflammatory hyperalgesia (Perrot et al., 1998). Peripheral inflammation was induced by a single subcutaneous injection of carrageenan (200 μl of 1% solution) into the plantar surface of the hind paw using a 30-gauge needle.

After formalin or carrageenan injections, microdialysis was carried out up to 3.5 or 6 h, respectively. The systemic treatment with MPEP (2 mg/kg i.p.) was performed 30 min before the subcutaneous injection of the noxious agents into the hind paw. Groups of rats received MPEP (1 mM) into dorsal raphe through the microdialytic probe before receiving formalin or carrageenan. Assuming that about 10% of MPEP diffuses from microdialysis probe to the surrounding tissue (Chaurasia, 1999), we chose a concentration of this drug 10 times that usually used in vitro studies (100 μM) (Kettunen et al., 2003; Marchetti et al., 2003; White et al., 2003). Thus, the total amount of MPEP locally released into the dorsal raphe results to be no higher than 100 μM corresponding to 0.5 μg. Finally, MPEP (30 nmol, 7 μg) was injected into the hind paw 15 min before administering formalin or carrageenan. To define a selective in vivo dose for MPEP, we performed extensive studies in our laboratory to determine the minimal dose required to antagonize agonist-induced effects.

2.3. Thermoceptive responses

Changes in thermoceptive responses have been evaluated according to Hargreaves et al. (1998) using a Plantar Test Apparatus (Ugo Basile, Varese, Italy). On the day of microdialysis, each animal was simultaneously placed in a plastic cage (22 × 17 × 14 cm, length × width × height) with a glass floor. After 1 h habituation period, the plantar surface of the hind paw was exposed to a beam of radiant heat through the glass floor. The radiant heat source consisted of an infrared bulb (Osram halogen-bellaphot bulb; 8 V, 50 W). A photoelectric cell detected light reflected from the paw and turned off the lamp when paw movement interrupted the reflected light. The paw withdrawal latency was automatically displayed to the nearest 0.1 s; the cut-off time was 25 s in order to prevent tissue damage. The latency of nociceptive reaction was measured in seconds each 30 min (within the time of changing the perfusate samples) under basal condition and after drug treatment and/or inducing pain. Each rat served as its own control, the latency to nociceptive response being measured both before and after treatments. At time 0, five baseline responses were already obtained for each animal at 30 min intervals and averaged. Groups of 10 animals per treatment were used, with each animal used for one treatment only.

2.4. Drugs

MPEP was purchased from Tocris Cookson, Bristol, UK, λ-carrageenan from Sigma and formalin from Carlo Erba. Formalin and carrageenan were dissolved in 0.9% NaCl. MPEP was dissolved in 0.9% NaCl for i.p. administration, in ACSF for direct intra-dorsal raphe delivery through the probe and in 100 mM HEPES (10 μl) for dorsal subcutaneous intra-hind paw administration.

2.5. Statistics

Results of microdialysis and plantar test were expressed as mean ± S.E.M. of the percentage of baseline (5-HT concentration or latency at a given point/mean baseline × 100) and data points plotted as the mean ± S.E.M.

Baseline values were calculated as the mean of five pre-drug sequential samples. Statistical analysis of the data was performed by analysis of variance at each point using one-way analysis of variance (ANOVA) followed by Newman–Keuls for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Microdialysis

The mean basal value (not corrected for probe recovery) of extracellular 5-HT levels in the dorsal raphe was 27 ± 4 fmol/20 μl (mean ± S.E.M.). This reported value of basal 5-HT was the mean concentration of all analysed rats.

3.1.1. Effect of formalin and carrageenan on dorsal raphe 5-HT release

Injection of formalin (5%, 50 μl) into the dorsum of the hind paw led, 30 min after formalin, to a significant ($P < 0.05$) increase in extracellular 5-HT (137 ± 5% of pre-injection baseline). The extracellular 5-HT increase lasted for 90 min after formalin injection, thereafter, the extracellular 5-HT levels turned again to the basal value (Fig. 1A). Intraplantar injection of carrageenan (1%, 200 μl) increased significantly ($P < 0.05$) extracellular concentration of 5-HT (212 ± 6% of pre-injection baseline) with a maximum effect 150 min after carrageenan injection (Fig. 1B).

3.1.2. Effect of MPEP on formalin-induced 5-HT increase

Pretreatment with MPEP (2 mg/kg i.p.), 30 min before formalin, induced a further and significant ($P < 0.05$) increase of 5-HT release (180 ± 11% of pre-injection base-

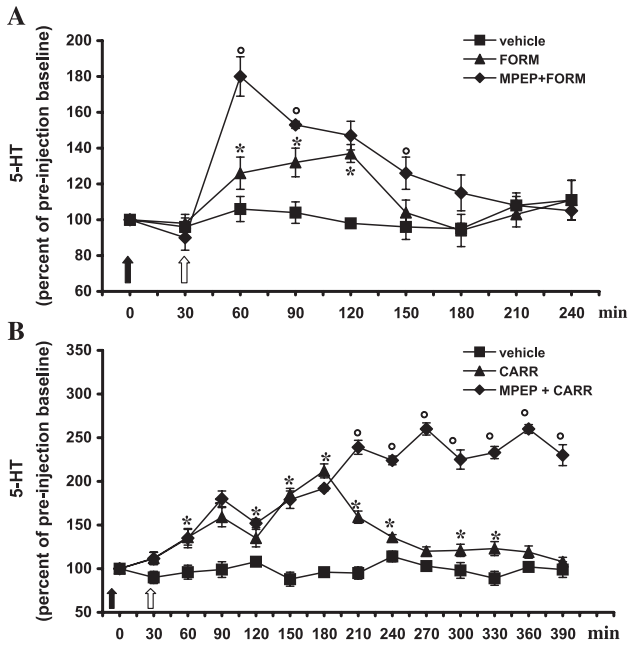

Fig. 1. Effects of vehicle (50 or 200 μl 0.9% saline), formalin (FORM, 50 μl 5%) (A) or carrageenan (CARR, 200 μl 1%) (B) injected into the hind paw in rats pretreated or not with MPEP (2 mg/kg i.p.) 30 min before FORM or CARR on dorsal raphe 5-HT extracellular concentration. The black arrows indicate the administration of MPEP and the white arrows indicate the injection of either FORM or CARR. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. FORM or CARR. *P*-values <0.05 were considered statistically significant.

line) (Fig. 1A). MPEP (1 mM, corresponding to 0.5 μg), directly infused into dorsal raphe during 30 min through microdialytic probe, did not modify significantly formalin-induced increase of dorsal raphe extracellular 5-HT levels (147 ± 10% of pre-injection baseline) (Fig. 2A). Another group of rats received MPEP (30 nmol, 7 μg) subcutaneously into the hind paw 15 min before formalin and this pretreatment significantly ($P<0.05$) prevented the formalin-induced increase of dorsal raphe extracellular 5-HT levels (108 ± 3% of pre-injection baseline) (Fig. 2B).

3.1.3. Effect of MPEP on carrageenan-induced 5-HT increase

The treatment with MPEP (2 mg/kg i.p.), 30 min before carrageenan, enhanced carrageenan-induced 5-HT release and the duration of the effect with the maximal increase (260 ± 12% of pre-injection baseline) 240 min after carrageenan (Fig. 1B). Carrageenan-induced increase of dorsal raphe extracellular 5-HT levels was not modified by MPEP perfused locally into dorsal raphe (189 ± 10% of pre-injection baseline) (Fig. 3A).

MPEP (30 nmol, corresponding to 7 μg) into the hind paw, 15 min before carrageenan, significantly ($P<0.05$) prevented carrageenan-induced increase of dorsal raphe extracellular 5-HT levels (126 ± 7% of pre-injection baseline) (Fig. 3B). Finally, MPEP (2 mg/kg i.p.), MPEP (1 mM, corresponding to 0.5 μg) directly delivered into dorsal raphe through the probe or MPEP (30 nmol, corresponding to 7 μg) into the hind paw did not significantly change extracellular dorsal raphe 5-HT (129 ± 11%, 121 ± 10% and 122 ± 7% of pre-injection baseline, respectively) (data not shown).

3.2. Thermoceptive responses

3.2.1. Effect of MPEP on formalin-induced hyperalgesia

Injection of 50 μl formalin 5% in the dorsum of the rat paw induced nociceptive behaviour (licking, shaking, lifting and occasionally biting of the injected forepaw) and reduced significantly ($P<0.05$) thermal withdrawal latency (23 ± 7% of pre-injection baseline) in the ipsilateral paw (Fig. 4A). Pretreatment with MPEP (2 mg/kg i.p.), 30 min before formalin, partially reversed ($P<0.05$) thermal hyperalgesia (71 ± 8% of pre-injection baseline) (Fig. 4A). No significant change on thermal hyperalgesia (35 ± 8% of pre-injection baseline) was observed when 1 mM MPEP was delivered into the dorsal raphe through microdialytic probe (Fig. 4B). Finally, MPEP (30 nmol, 7 μg) into the hind paw, 15 min before formalin, prevented (108 ± 3% of pre-injection baseline) formalin-induced thermal hyperalgesia (Fig. 5A). No change in the latency of nociceptive reaction, with or

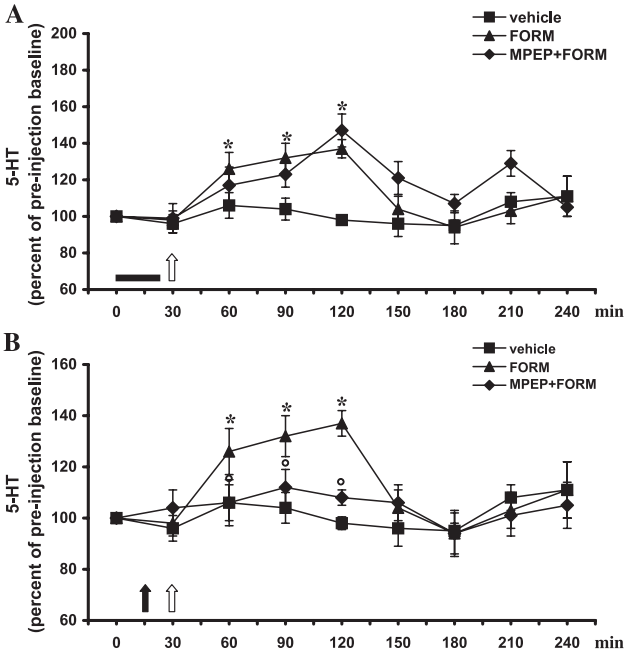

Fig. 2. Effects of vehicle (50 μl 0.9% saline) or formalin (FORM, 50 μl 5%) injected into the hind paw in rats pretreated or not with MPEP (1 mM) infused into dorsal raphe through a microdialysis probe for 30 min before FORM (A) or MPEP (30 nmol) injected into the dorsal surface of the hind paw 15 min before FORM (B) on dorsal raphe 5-HT extracellular concentration. The black bar indicates the time of perfusion with MPEP, the white arrows show the injection of FORM and the black arrow the peripheral injection of MPEP. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Shows significant differences vs. vehicle and (°) significant differences vs. FORM. *P*-values <0.05 were considered statistically significant.

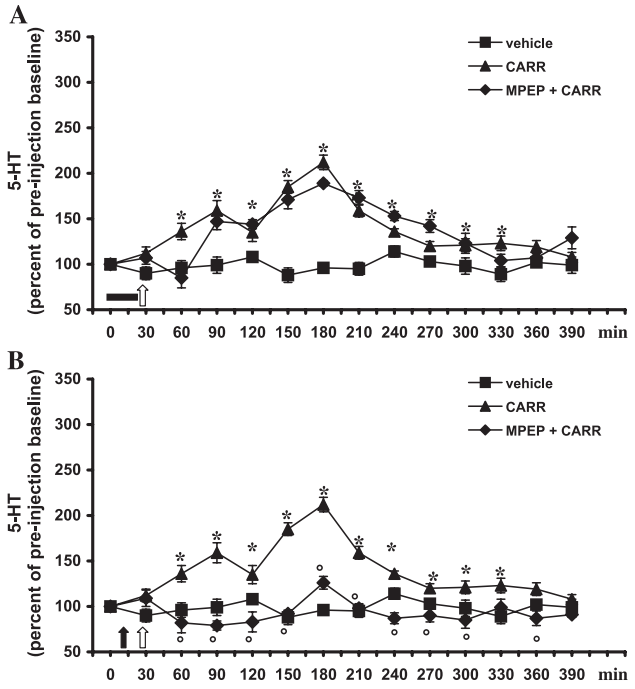

Fig. 3. Effects of vehicle (200 μl 0.9% saline) or carrageenan (CARR, 200 μl 1%) injected into the hind paw in rats pretreated either with MPEP (1 mM) infused into dorsal raphe through a microdialysis probe for 30 min before CARR (A) or with MPEP (30 nmol) injected into the dorsal surface of the hind paw 15 min before CARR (B) on dorsal raphe 5-HT extracellular concentration. The black bar indicates the perfusion with MPEP, the white arrows indicate the injection of CARR and the black arrow indicates the peripheral injection of MPEP. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. CARR. *P*-values < 0.05 were considered statistically significant.

without pretreatment with MPEP, was observed in the contralateral paw (data not shown).

3.2.2. Effect of MPEP on carrageenan-induced hyperalgesia

Intraplantar injection of carrageenan (1%, 200 μl) significantly ($P < 0.05$) reduced thermal threshold (19 ± 3% of pre-injection baseline) (Fig. 5B). Systemic pretreatment with MPEP (2 mg/kg i.p.) reduced thermal hyperalgesia (80 ± 10% pre-injection of baseline) (Fig. 5B) but did not change it (25 ± 9% of pre-injection baseline) when locally delivered through microdialytic probe at 1 mM concentration (Fig. 6A). Finally, when MPEP (30 nmol/rat) was administrated into the hind paw, the carrageenan-induced thermal hyperalgesia was reduced (117 ± 7% of pre-injection baseline) (Fig. 6B). No significant changes in the latency of nociceptive reaction, with or without pretreatment with MPEP, have been observed into the contralateral paw (data not shown) following carrageenan injection.

3.3. Animal behaviour

No overt behavioural changes were observed in this study following administration of MPEP, with all animals remaining alert but generally inactive throughout the experiment. Indeed, MPEP has been used previously under similar experimental conditions and it never modified locomotor activity (Walker et al., 2001a).

4. Discussion

A body of evidence shows that 5-HT is released at spinal (Godefroy et al., 1987; Schoenen et al., 1985; Tyce and Yaksh, 1981) and supraspinal levels (Zhang et al., 2000) following pain. At present, however, there are no intensive studies aimed at monitoring any change of 5-HT release into the dorsal raphe, where the bulk of serotonergic connection fibres with pain perceptive and/or pain modulating nuclei originate (Oliveras et al., 1979; Wang and Nakai, 1994). Moreover, dorsal raphe contains not only the cell bodies and dendrites of serotonergic neurons but also an intricate network of 5-HT fibres. In the current study, we have investigated changes of dorsal raphe 5-HT release in formalin and carrageenan models of inflammatory pain. Furthermore, the effect of $mGlu_5$ receptors blockade at different sites, in these pain conditions, has been investigated to clarify the biochemical changes and behavioural thermoceptive responses associated to MPEP, the latter having shown interesting

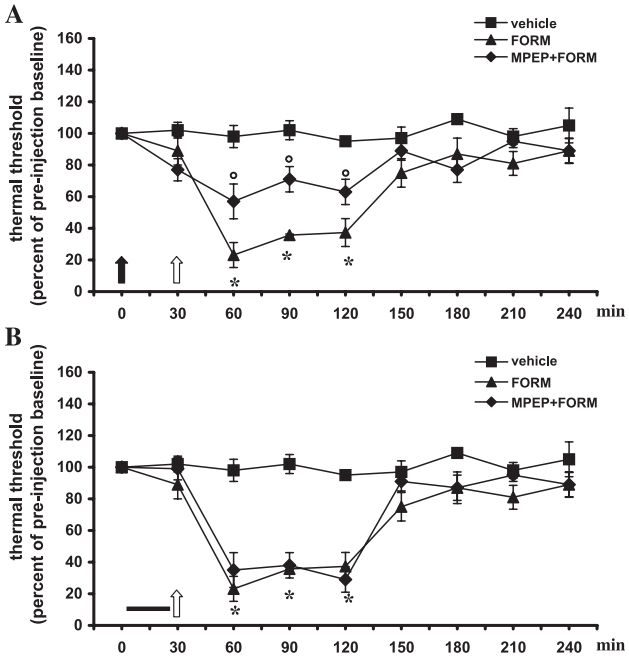

Fig. 4. Effects of vehicle (50 μl 0.9% saline) or formalin (FORM, 50 μl 5%) injected into the hind paw in rats pretreated or not with MPEP (2 mg/kg i.p.) 30 min before FORM (A) or MPEP (1 mM) infused into dorsal raphe through a microdialysis probe for 30 min before FORM (B) on thermal withdrawal latency. The black arrow indicates the intraperitoneal injection of MPEP, the white arrows indicate the injection of FORM and the black bar indicates the perfusion with MPEP. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. FORM. *P*-values < 0.05 were considered statistically significant.

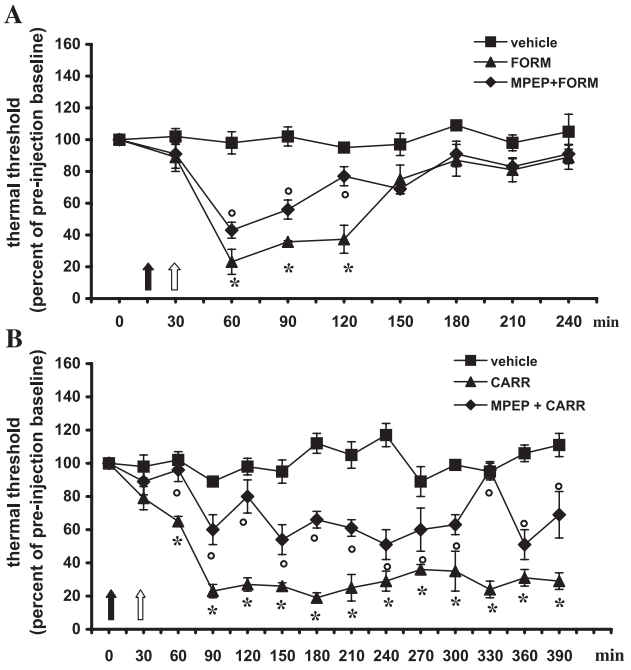

Fig. 5. Effects of vehicle (50 μl 0.9% saline) or formalin (FORM, 50 μl 5%) injected into the hind paw in rats pretreated or not with MPEP (30 nmol) into the dorsal surface of the hind paw 15 min before FORM on thermal withdrawal latency (A). Effects of vehicle (200 μl 0.9% saline) or carrageenan (CARR, 200 μl 1%) injected into the hind paw in rats pretreated or not with MPEP (2 mg/kg i.p.) 30 min before CARR on thermal withdrawal latency (B). The black arrows indicate the peripheral or intraperitoneal injection with MPEP and the white arrows the injections of FORM or CARR. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. FORM or CARR. *P*-values <0.05 were considered statistically significant.

analgesic properties in inflammatory hyperalgesia (Walker et al., 2001a,b).

Subcutaneous formalin, in common with previous studies related to other centres of the endogenous pain inhibitory system (Fasmer et al., 1985; Puig et al., 1992; Omote et al., 1998), increased dorsal raphe extracellular 5-HT levels. Similarly to formalin, intraplantar carrageenan increased dorsal raphe 5-HT. Even if both formalin and carrageenan have been used to induce inflammatory pain states, we do not ignore the different onset and time course of nociceptive inputs and responses as well as the different neurochemical changes contributing to hyperalgesia (Honoré et al., 1999). It is interesting, however, to observe that in this study 5-HT increase is consistent with early developing periods of tonic pain and with the long lasting hyperalgesia, which peaks 2–3 h after carrageenan injection.

Dorsal raphe serotonergic neurons happen to be activated following a nociceptive stimulus, which is consistent with massive release. This increase of 5-HT release following inflammatory pain has been already shown into the spinal cord and into the periaqueductal gray, an area with functional and morphological properties close to dorsal raphe (Zhang et al., 2000). These authors demonstrated that such increase in 5-HT release was due to an opioid-mediated decrease of γ-aminobutyric acid (GABA) tone leading to a disinhibitory action on serotonergic neurons. As already mentioned, apart from cell bodies and dendrites, dorsal raphe contains an intricate network of 5-HT fibres where vesicular release occurs.

It is known that $mGlu_5$ receptors, which are distributed on the whole neuroaxis of the somatosensory pathway, are implicated in nociceptive processes (Martin et al., 1992; Shigemoto and Mizuno, 2000). Due to the key role of dorsal raphe 5-HT in inhibiting pain and to the analgesic property of MPEP, we expected that serotonergic activity would be enhanced by MPEP. Indeed, peripheral, spinal and cerebral selective blockade of $mGlu_5$ receptors by MPEP can reduce nociceptive responses with a certain kind of variability owing to the relative localization on a variety of pre- and postsynaptic elements of GABA or non-GABA containing neurons, and specific pain types in the rat (Dogrul et al., 2000; Bhave et al., 2001; Walker et al., 2001a).

As expected, systemic administration of MPEP, before the noxious stimulus, produced an enhancement in dorsal raphe 5-HT release and partially reversed thermal hyper-

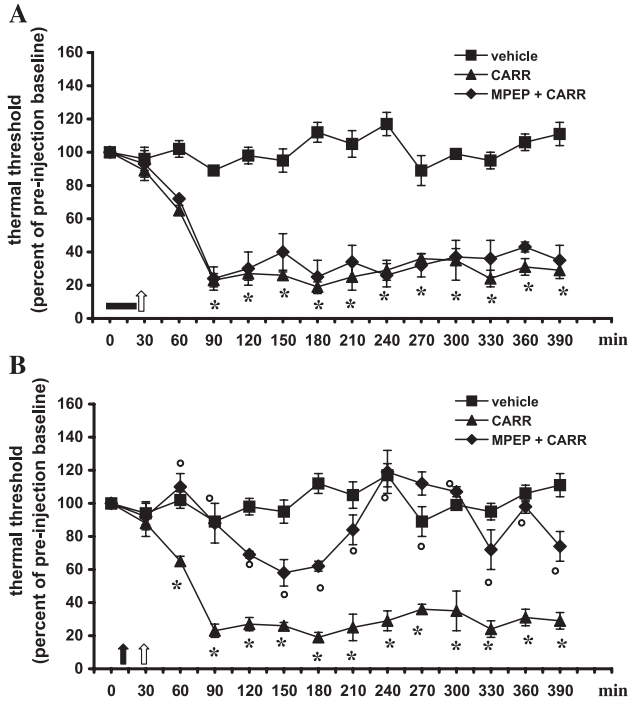

Fig. 6. Effects of vehicle (200 μl 0.9% saline) or carrageenan (CARR, 200 μl 1%) injected into the hind paw in rats pretreated or not with MPEP (1 mM) infused into dorsal raphe through a microdialysis probe for 30 min before CARR (A) or MPEP (30 nmol) injected into the dorsal surface of the hind paw 15 min before CARR (B) on thermal withdrawal latency. The black bar indicates the time of perfusion with MPEP, the white arrows the injection with CARR and the black arrow the peripheral injection with MPEP. Each point represents the mean ± S.E.M. of 10 animals per group. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. CARR. *P*-values <0.05 were considered statistically significant.

algesia in these models of inflammatory pain. The further enhancement of dorsal raphe 5-HT release by systemic pretreatment and its analgesic property give no evidence of whether central or peripheral $mGlu_5$ receptors are involved. To verify if 5-HT increase enhancement by systemic MPEP was due to a local dorsal raphe $mGlu_5$ receptors blockade, MPEP was directly perfused into the dorsal raphe through microdialytic probes in both models of inflammatory pain. This treatment failed to modify significantly the pain-induced 5-HT increase and to change thermal hyperalgesia. This seems to exclude a direct serotonergic facilitation played by $mGlu_5$ receptors at dorsal raphe level. Interestingly, centrally administered MPEP (intrathecal or intracerebroventricular) has shown not to change Freund's complete adjuvant- induced mechanical hyperalgesia (Walker et al., 2001a). Peripheral $mGlu_5$ receptors mediate a pivotal role in the nerve sensitisation, which is linked with inflammatory conditions. The blockade of peripheral $mGlu_5$ sensory afferents before the noxious insult should prevent the development of any process leading to inflammatory pain (Bhave et al., 2001). To further confirm this issue, MPEP was subcutaneously injected into the rat hind paw before administering formalin or carrageenan. In this case, MPEP abolished formalin and carrageenan-induced dorsal raphe 5-HT increase and thermal hyperalgesia. A plausible mechanism is that $mGlu_5$ receptors in sensory neurons, activate via prostaglandins production, a vanilloid type 1 receptor (VR1), which is a key mediator of inflammatory thermal hyperalgesia (Hu et al., 2002). MPEP delivered subcutaneously into the hind paw may block peripheral $mGlu_5$ preventing such a component of inflammatory mechanisms which led to pain-induced increase in 5-HT release and thermal hyperalgesia (Bhave et al., 2001; Hu et al., 2002). In fact, there is evidence that glutamate, released in the periphery after inflammation, is a key mediator of inflammation-evoked hyperalgesia (deGroot et al., 2000).

MPEP, per se, did not modify 5-HT release and thermal nociception either through systemic administration, or with intra-dorsal raphe local perfusion, or through intra-paw administration. Since the $mGlu_5$ receptor is involved in the induction and maintenance of peripheral sensitisation, the latter being a component of inflammatory pain, it is not surprising that MPEP was effective in changing dorsal raphe 5-HT release and in reversing hyperalgesia only in this condition. Although, the participation of $mGluR_5$ located in other nociceptive strategic circuits (i.e. spinal dorsal horn, rostral ventrolateral medulla, central amygdala, etc.) may be not excluded, this study shows that dorsal raphe $mGlu_5$ receptors do not tonically modulate 5-HT release while extracellular 5-HT values were increased at that level by inflammatory pain conditions. The pain-induced release of 5-HT was modified only through peripheral $mGlu_5$ glutamate receptors blockade, which simultaneously reduced inflammation-induced thermal hyperalgesia.

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