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Assessing the role of metabotropic glutamate receptor 5 in multiple nociceptive modalities

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Abstract

Preclinical data, performed in a limited number of pain models, suggest that functional blockade of metabotropic glutamate (mGlu) receptors may be beneficial for pain management. In the present study, effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective mGlu5 receptor antagonist, were examined in a wide variety of rodent nociceptive and hypersensitivity models in order to fully characterize the potential analgesic profile of mGlu5 receptor blockade. Effects of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), as potent and selective as MPEP at mGlu5/mGlu1 receptors but more selective than MPEP at *N*-methyl-aspartate (NMDA) receptors, were also evaluated in selected nociceptive and side effect models. MPEP (3–30 mg/kg, i.p.) produced a dose-dependent reversal of thermal and mechanical hyperalgesia following complete Freund's adjuvant (CFA)-induced inflammatory hypersensitivity. Additionally, MPEP (3–30 mg/kg, i.p.) decreased thermal hyperalgesia observed in carrageenan-induced inflammatory hypersensitivity without affecting paw edema, abolished acetic acid-induced writhing activity in mice, and was shown to reduce mechanical allodynia and thermal hyperalgesia observed in a model of post-operative hypersensitivity and formalin-induced spontaneous pain. Furthermore, at 30 mg/kg, i.p., MPEP significantly attenuated mechanical allodynia observed in three neuropathic pain models, i.e. spinal nerve ligation, sciatic nerve constriction and vincristine-induced neuropathic pain. MTEP (3–30 mg/kg, i.p.) also potently reduced CFA-induced thermal hyperalgesia. However, at 100 mg/kg, i.p., MPEP and MTEP produced central nerve system (CNS) side effects as measured by rotarod performance and exploratory locomotor activity. These results suggest a role for mGlu5 receptors in multiple nociceptive modalities, though CNS side effects may be a limiting factor in developing mGlu5 receptor analgesic compounds.

Keywords: MPEP; MTEP; Glutamate; Hyperalgesia; Allodynia; Modality

1. Introduction

Glutamate is the predominant excitatory neurotransmitter of the central and peripheral nervous system. Glutamatergic activity is mediated via ligand gated ionotropic glutamate (iGlu) receptor and G-protein coupled metabotropic glutamate (mGlu) receptors (Bordi and Ugolini, 1999). mGlu receptors modulate neuronal excitability and synaptic transmission and are divided into three groups (I, II, and III) based on pharmacology, signal transduction and sequence homology. Group I mGlu receptors, comprising mGlu1 and mGlu5 receptors, couple primarily to induce the stimulation of phospholipase C, the release of Ca^{2+} from intracellular stores, and the activation of protein kinase C (Crawford et al., 2000). Group I mGlu receptors, particularly mGlu5 receptors, have been implicated in nociceptive transmission, given the wide expression of mGlu5 receptors along the nociception-relevant somatosensory neuraxis (Neugebauer, 2002). Correspondingly, glutamate can be released at various level of the nociceptive pathways in response to inflammation, tissue injury or (*RS*)-dihydroxy phenylglycine (DHPG), a selective

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group I mGlu receptor agonist application, while mGlu5 receptor antagonists can block such release at both peripheral and spinal cord levels (Lorrain et al., 2002; Omote et al., 1998; Zahn et al., 2002). In addition, electrophysiological studies have demonstrated that mGlu5 receptor agonists induce activation of spinal cord and thalamic neurons, that can be prevented by mGlu5 receptor antagonists (Salt et al., 1999; Walker et al., 2001b).

Behavioral studies have shown that spinal administration of DHPG produces thermal hyperalgesia, mechanical allodynia, spontaneous nociceptive behaviors, and facilitates formalin-induced nociception in rats (Dolan and Nolan, 2000; Fisher and Coderre, 1998; Lorrain et al., 2002). In addition, intraplantar (i.pl.) administration of DHPG has also been shown to induce thermal hyperalgesia in mice (Bhave et al., 2001). Correspondingly, intrathecal (i.t.) administration of antibodies or antagonists to group I mGlu receptors reduced nociceptive behaviors in chronic constriction injury of the sciatic nerve in rats (Fisher et al., 1998; Fundytus et al., 1998). In addition, i.pl. administration of SIB-1757 (6-methyl-2-(phenylazo)-pyridin-3-ol), the first subtype-selective mGlu5 receptor antagonist, fully reversed spinal nerve ligation-induced thermal hyperalgesia in rats (Dogrul et al., 2000); however, this compound is a weak mGlu5 receptor antagonist (IC_{50}=370 nM) (Varney et al., 1999).

Discovery of potent and highly subtype-selective mGlu5 receptor antagonists, 2-methyl-6-(phenylethynyl)-pyridine (MPEP, IC_{50}=2 nM), and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP, IC_{50}=5 nM) (Cosford et al., 2003) has benefited further evaluation of the specific role of the mGlu5 receptors in nociceptive transmission and modulation. Oral administration of MPEP has previously been shown to produce analgesia in a limited number of pain models, e.g. mechanical hyperalgesia in inflammatory hypersensitivity models measured by paw pressure and thermal hyperalgesia in spinal nerve ligation-induced neuropathic pain in rats (Hudson et al., 2002; Walker et al., 2001a), while effects of the more recent MTEP in in vivo pain models have not yet been described. In the present study, the effects of MPEP were examined in a wide variety of inflammatory, neuropathic, visceral and post-operative nociceptive models, in order to fully characterize the potential analgesic profile of selective blockade of mGlu5 receptors. In addition, the effects of MPEP were also evaluated in locomotor side effect assays to rule out potential analgesic confounds. Furthermore, MTEP was reported to be as potent and selective in in vitro functional assay as MPEP at mGlu5/mGlu1 receptors, but more selective versus *N*-methyl-aspartate (NMDA) receptors (Cosford et al., 2003), suggesting that it could potentially produce fewer central nerve system (CNS) side effects. Therefore, the effects of MTEP were also evaluated in selective nociceptive and locomotor side effect models to further examine the role of mGlu5 receptors in nociceptive modulation.

2. Material and methods

2.1. Animals and compounds

Adult male Sprague–Dawley rats (200–300 g) and CD1/ICR mice (20–25 g) (Charles River Laboratories, Portage, MI) were used in this study. Animal handling and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Abbott Laboratories. For all surgical procedures, animals were maintained under halothane anesthesia (4% to induce, 2% to maintain), and the incision sites were sterilized using a 10% povidone-iodine solution prior to and post surgeries.

MPEP and MTEP were synthesized at Abbott Laboratories as previously described (Cosford et al., 2003; Gasparini et al., 1999b) (molecular weight of 229.7, 200.3, respectively), and were administered intraperitoneally (i.p.) in all experiments. Vincristine sulfate, dimethyl sulfoxide (DMSO) and hydroxypropyl-β-cyclodextrin (HBC) were obtained from Sigma (St. Louis, MO). For all rat experiments, MPEP and MTEP were dissolved in a solution of DMSO/HBC/sterile water (10:33:57, V/V) in a final volume of 2 ml/kg. For mouse visceral pain experiments, MPEP was dissolved in a vehicle consisting of DMSO/HBC/sterile water (3:6:91, V/V) in a final volume of 10 ml/kg.

2.2. Inflammatory pain models

2.2.1. Formalin test

The formalin assay was performed following the procedure originally described by Dubuisson and Dennis (1977); 50 μl of a 5% formalin solution was injected subcutaneously (s.c.) into the dorsal aspect of the right hind paw and the rats were then individually placed into clear observation cages. Rats were observed for a continuous period of 60 min or for periods of time corresponding to phase I (from 0 to 10 min following formalin injection) and phase II (from 30 to 50 min following formalin injection) of the formalin test (Abbott et al., 1995). The number of flinching behaviors of the injected paw was recorded using a sampling technique in which each animal was observed for one 60-s period during each 5-min interval; thus twelve 60-s observation periods were recorded. MPEP (3, 10, 30 mg/kg, i.p., *n*=6) was administered 30 min prior to formalin injection.

2.2.2. Carrageenan-induced acute thermal hyperalgesia and edema

Paw edema and acute thermal hyperalgesia were induced by injecting 100 μl of a 1% solution of λ-carrageenan(Sigma) in physiological saline into the plantar surface of the right hind paw. Thermal hyperalgesia was determined 2 h following carrageenan injection, using a commercially available thermal paw stimulator (UARDG, University of California, San Diego, CA) as described by Hargreaves et al. (1988). Rats were placed into individual plastic cubicles mounted on a glass surface maintained at 30 °C, where a

thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, was then applied to the plantar surface of each hind paw. The stimulus current was maintained at 4.50±0.05 amp, and the maximum time of exposure was set at 20.48 s to limit possible tissue damage. The elapsed time until a brisk withdrawal of the hind paw from the thermal stimulus was recorded automatically using photodiode motion sensors. The right and left hind paw of each rat was tested in three sequential trials at approximately 5-min intervals. Carrageenan-induced thermal hyperalgesia of paw withdrawal latency ($PWL_{thermal}$) was calculated as the mean of the two shortest latencies. MPEP (3, 10, 30 mg/kg, i.p., n=6) was administered 30 min before test for thermal hyperalgesia.

The volume of paw edema was measured using water displacement with a plethysmometer (Buxco, Sharon, CT) 2 h following carrageenan injection, by submerging the paw up to the ankle hairline (approximately 1.5 cm). The displacement of the volume was measured by a transducer and recorded by a computer. MPEP (3, 10, 30 mg/kg, i.p., n=8) was administered either 30 min prior to, or 90 min following carrageenan injection.

2.2.3. Complete Freund's adjuvant (CFA)-induced chronic thermal and mechanical hyperalgesia

Chronic inflammatory thermal and mechanical hyperalgesia were induced by injection of 150 μl of a 50% solution of CFA in phosphate buffered saline (PBS) into the plantar surface of the right hind paw in rats; control animals received only PBS treatment. $PWL_{thermal}$ was assessed 48 h following CFA injection in these animals in a manner similar to that described above for carrageenan-injected animals. MPEP, MTEP (3, 10, 30 mg/kg, i.p., n=6) was administered 30 min before test for thermal hyperalgesia.

CFA-induced mechanical hyperalgesia was examined 48 h following CFA injection, using the Randall Selitto test as previously described (Stein et al., 1988). This paw pressure analgesia instrument (Stoelting, Wood Dale, IL) employs an increasing pressure on the rat hind paw between a flat surface and a blunt pointer of a sharp wedge probe, until the animal makes a stereotyped withdrawal response. The reading of the force indicator at the end point was recorded as the paw withdrawal threshold ($PWT_{Randall}$). MPEP (3, 10, 30 mg/kg, i.p., n=8) was administered 30 min before test for mechanical $PWT_{Randall}$ in both the CFA-treated and uninjected paw.

2.3. Visceral pain

Thirty minutes following MPEP (3, 10, 30 mg/kg, i.p., n=8) administration, mice received an injection of 0.6% acetic acid in sterile water (10 ml/kg, i.p.) as previously described (Mogil et al., 1999). Mice were then placed in individual table-top plexiglass observation cylinders (60 cm high; 40 cm diameter) where the number of constrictions/writhes (a wave of mild constriction and elongation passing caudally along the abdominal wall, accompanied by a slight twisting of the trunk and followed by bilateral extension of the hind limbs) was recorded during the 5–20 min following acetic acid injection for a continuous observation period of 15 min.

2.4. Neuropathic pain

2.4.1. Spinal nerve ligation

Rats received unilateral ligation of the lumbar 5 (L5) and lumbar 6 (L6) spinal nerves as previously described (Kim and Chung, 1992). The left L5 and L6 spinal nerves of the rat were isolated adjacent to the vertebral column and tightly ligated with a 5-0 silk suture distal to the dorsal root ganglia, and care was taken to avoid injury of the lumbar 4 (L4) spinal nerve. Sham rats underwent the same procedure, but without nerve ligation. All animals were allowed to recover for at least 1 week and not more than 3 weeks prior to assessment of mechanical allodynia. Mechanical allodynia was measured using calibrated von Frey filaments (Stoelting, Wood Dale, IL) as previously described (Chaplan et al., 1994). Rats were placed into inverted individual plastic containers (20×12.5×20 cm) on top of a suspended wire mesh grid, and acclimated to the test chambers for 20 min. The von Frey filaments were presented perpendicularly to the plantar surface of the selected hind paw, and then held in this position for approximately 8 s with enough force to cause a slight bend in the filament. Positive responses included an abrupt withdrawal of the hind paw from the stimulus, or flinching behavior immediately following removal of the stimulus. A 50% paw withdrawal threshold ($PWT_{von\ Frey}$) was determined using an up–down procedure (Dixon, 1980). Only rats with a $PWT_{von\ Frey} \leq 5.0$ g were considered allodynic and utilized to test the analgesic activity of MPEP (3, 10, 30 mg/kg, i.p., n=6). The compound was administered 30 min prior to the assessment of mechanical allodynia.

2.4.2. Chronic constriction injury of the sciatic nerve

A model of chronic constriction injury of the sciatic nerve-induced neuropathic pain in rats was produced by following the method of Bennett and Xie (1988). The right common sciatic nerve was isolated at mid-thigh level, and loosely ligated by four chromic gut (4-0) ties separated by an interval of 1 mm. Sham rats underwent the same procedure, but without sciatic nerve constriction. All animals were allowed to recover for at least 2 weeks and no more than 5 weeks prior to testing of mechanical allodynia. Allodynic $PWT_{von\ Frey}$ was assessed in these animals in a manner similar to that described above for animals with spinal nerve ligation. Only rats with a $PWT_{von\ Frey} \leq 5.0$ g were considered allodynic and utilized to test the analgesic activity of MPEP (3, 10, 30 mg/kg, i.p., n=6). The compound was administered 30 min prior to the assessment of mechanical allodynia.

2.4.3. Vincristine-induced mechanical allodynia

A model of chemotherapy-induced neuropathic pain was produced by continuous intravenous (i.v.) vincristine infusion (Nozaki-Taguchi et al., 2001). In this model, anesthetized rats underwent a surgical procedure consisting of jugular vein catheterization and subcutaneous implantation of a vincristine-primed mini-pump. Fourteen days of i.v. infusion of vincristine (30 μg/kg/day) in physiological saline resulted in systemic neuropathic pain of the animal. Sham rats underwent the same procedure, but with physiological saline infusion. $PWT_{von\ Frey}$ of the left paw was assessed in these animals 14 days post implantation of mini-pumps in a manner similar to that described above for animals with spinal nerve ligation. MPEP (3, 10, 30 mg/kg, i.p., n=6) was administered 30 min prior to the test for mechanical allodynia in rats with $PWT_{von\ Frey} \leq 5.00$ g before treatment.

2.5. Post-operative pain

A model of post-operative pain was performed in rats as described by Brennan et al. (1996). The plantar aspect of the left hind paw was exposed through a hole in a sterile plastic drape, and a 1-cm longitudinal incision was made through the skin and fascia, starting 0.5 cm from the proximal edge of the heel and extending towards the toes. The plantaris muscle was elevated and incised longitudinally leaving the muscle origin and insertion points intact. After hemostasis by application of gentle pressure, the skin was apposed with two mattress sutures using 5-0 nylon. Animals were then allowed to recover for 2 h following surgery, at which time mechanical allodynia and thermal hyperalgesia were assessed.

Effects of MPEP (3, 10, 30 mg/kg, i.p., n=6) on mechanical allodynia were assessed 30 min following MPEP administration, with $PWT_{von\ Frey}$ being examined in these animals for both the injured and non-injured paw in a manner similar to that described above for animals with spinal nerve ligation, the von Frey filament systematically pointing towards the medial side of the incision (Brennan et al., 1996). No baseline withdrawal threshold was defined in this experiment, since all animals with skin incision developed hypersensitivity to von Frey filament stimulation.

In a separate experiment, the effects of MPEP (3, 10, 30 mg/kg, i.p., n=6) on thermal hyperalgesia were assessed 30 min following MPEP administration, with $PWL_{thermal}$ being determined in a manner similar to that described in carrageen-induced thermal hyperalgesia, the thermal stimulus applied to the center of the incision on the paw planter aspect.

2.6. Locomotor activity and motor coordination

2.6.1. Locomotor activity

Locomotor activity was measured in an open field using photobeam activity monitors (AccuScan Instruments, Columbus, OH). Rats were placed into 42×42×30 cm activity chambers where photobeam breaks were recorded for 30 min. MPEP (10, 30, 100 mg/kg, i.p., n=8) or MTEP (10, 30, 100 mg/kg, i.p., n=4) was administered 30 min prior to the assessment of locomotor activity.

2.6.2. Motor coordination

Motor coordination was measured using an accelerating rotarod apparatus (Omnitech Electronics, Columbus, OH). Rats were placed onto a 9-cm diameter rod, which increased in speed from 0 to 20 r.p.m. over a 60-s period. The time required for the rat to fall from the rod was automatically recorded, with a maximum cut-off of 60 s.

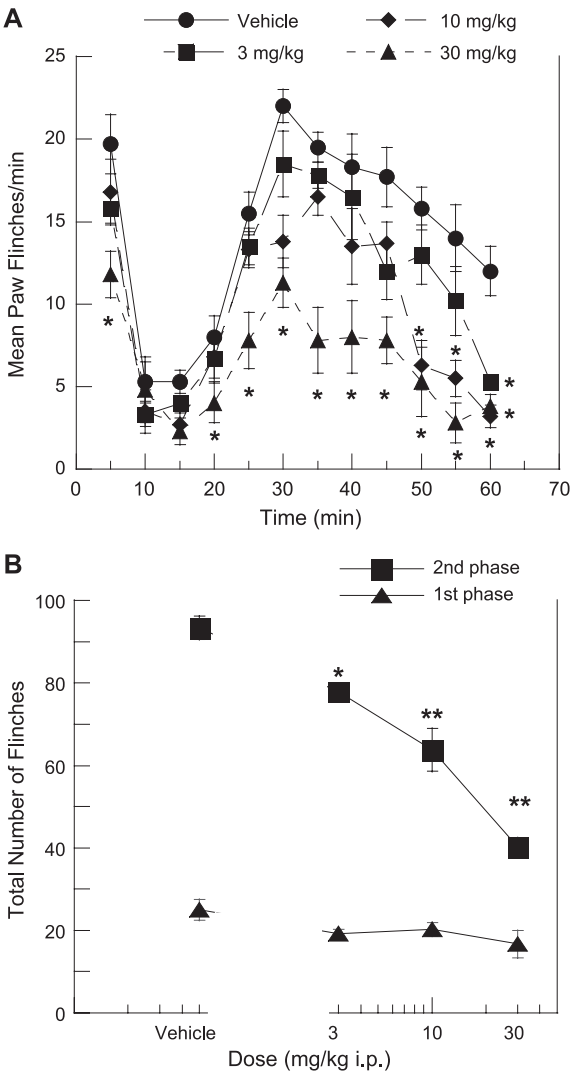

Fig. 1. Effects of MPEP on formalin-induced spontaneous pain in the rat. (A) A 50 μl of 5% formalin injection induced a characteristic biphasic flinching response over a 60-min observation period, MPEP at 30 mg/kg reduced paw flinches at every time point observed, except 10, 15 min, following formalin injection. (B) MPEP dose-dependently reduced formalin-induced phase II, but not phase I flinching behaviors, when data was expressed as phase I (0–10 min) and phase II (30–50 min following formalin injection). MPEP was administered 30 min before formalin injection. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group (n=6).

Table 1
MPEP analgesic profile

Pain models (stimulus)	Percent effect at 30 mg/kg, i.p.	ED_{50} (mg/kg, i.p.)
Rat formalin (phase II)	57	20
Rat carrageenan (thermal hyperalgesia)	50	30
Rat CFA (thermal hyperalgesia)	93	7
Rat CFA (mechanical hyperalgesia)	92	15
Mouse writhing	81	15
Rat spinal nerve ligation (mechanical allodynia)	33	>30
Rat sciatic nerve injury (mechanical allodynia)	39	>30
Rat vincristine (mechanical allodynia)	40	>30
Rat skin incision (thermal hyperalgesia)	50	30
Rat skin incision (mechanical allodynia)	35	>30

Results are presented as percent effect at 30 mg/kg, i.p. and ED_{50} (mg/kg, i.p.).

Following three training sessions, rats were randomly assigned into treatment groups. Latency to fall from the rotarod was determined 30 min following MPEP or MTEP (10, 30, 100 mg/kg, i.p., n=8) administration, and these values were used for statistical comparisons.

2.7. Statistics

Data are presented as mean±S.E.M., statistical significance was evaluated using analysis of variance (ANOVA) followed by Fisher's protected least significant difference (Fisher's PLSD) for post hoc analysis of multiple comparisons (GB-stat, Dynamic Microsystems); level of significance was set at P<0.05. The dose required to produce a 50% effect (ED_{50}) in each model and each stimulation condition was extrapolated from the dose–response curves using KaleidaGraph (Synergy Software).

3. Results

3.1. Effects of MPEP on inflammatory pain

3.1.1. Formalin test

Right hind paw injection of 5% formalin induced a characteristic biphasic flinching response. MPEP at 30 mg/kg, i.p. significantly reduced the number of flinches at every time point measured, except 10 and 15 min following formalin injection. MPEP at 10 mg/kg, i.p. significantly reduced paw flinches measured at 50–60 min following formalin injection (Fig. 1A). When data were expressed as phase I (0–10 min) or phase II (30–50 min), MPEP (3, 10, 30 mg/kg, i.p.) dose dependently reduced flinching response by 17%, 32% and 57%, (P<0.05, 0.01, 0.01, respectively, ED_{50}=20 mg/kg, i.p.) during phase II but had no significant effects on phase I (Fig. 1B, Table 1).

3.1.2. Carrageenan-induced thermal hyperalgesia and paw edema

Following carrageenan injection into the hind paw, $PWL_{thermal}$ was significantly reduced (3.34±0.26 s) compared to the contralateral hind paw (11.80±0.30 s) or saline-treated control animals (11.20±0.30 s). MPEP (3, 10, 30 mg/kg, i.p.) dose-dependently increased $PWL_{thermal}$ in carrageenan-injected paw when administered 30 min before test for thermal hyperalgesia (ED_{50}=30 mg/kg, i.p.) while having no significant effect

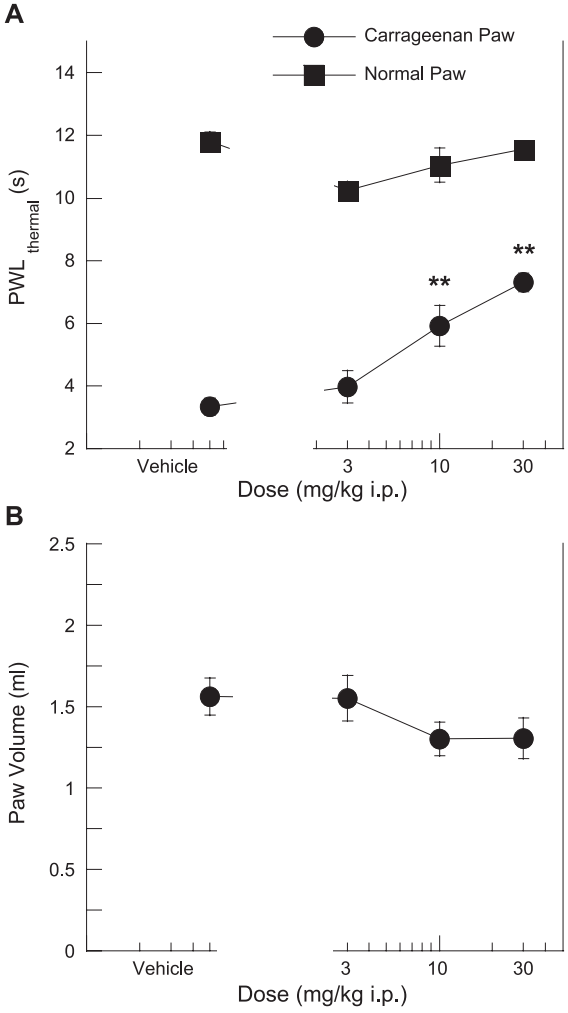

Fig. 2. Effects of MPEP on carrageenan-induced thermal hyperalgesia and paw edema in the rat. (A) MPEP, administered 90 min following carrageenan injection, dose-dependently increased $PWL_{thermal}$ of carrageenan-injected paw without affecting $PWL_{thermal}$ of the contralateral paw measured 2 h following carrageenan injection. (B) MPEP, administered 90 min following carrageenan injection, failed to reduce carrageenan-induced paw edema. Paw volume was measured 2 h following carrageenan injection. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group (n=6).

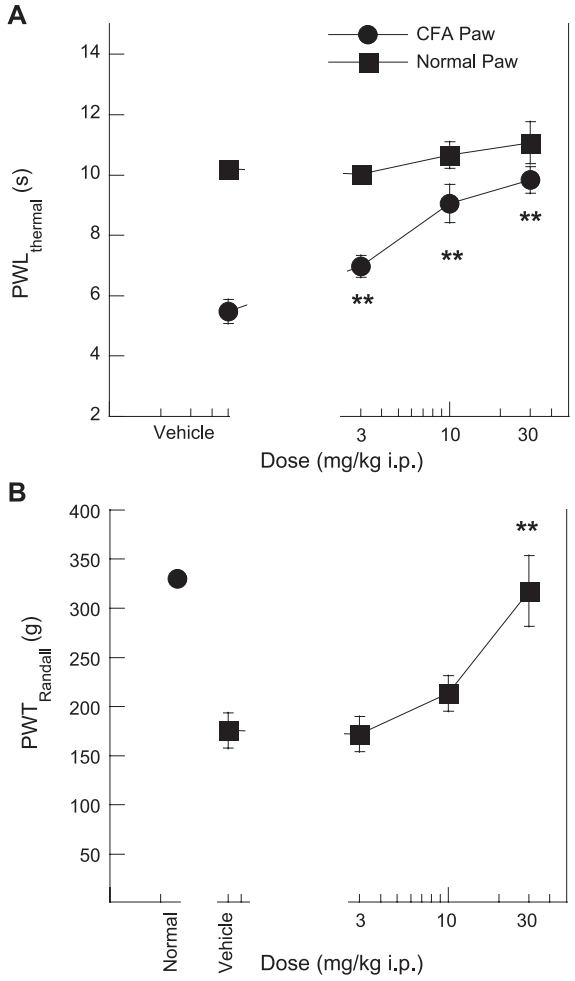

Fig. 3. Effects of MPEP on CFA-induced thermal and mechanical hyperalgesia in the rat. (A) MPEP dose-dependently increased $PWL_{thermal}$ of the CFA-injected paw to normal level, without affecting $PWL_{thermal}$ of the contralateral paw (n=6). (B) MPEP dose-dependently increased mechanical $PWT_{Randall}$ of the CFA-injected paw to normal level in a paw pressure test (n=8). MPEP was administered 30 min before nociceptive behavioral assessment. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group.

on contralateral $PWL_{thermal}$ (Fig. 2A, Table 1). No significant effect on carrageenan-induced edema was observed in a separate group of animals when MPEP (3, 10, 30 mg/kg, i.p.) was administered 30 min before edema measurement (Fig. 2B). In addition, no effects on edema were observed when MPEP (3, 10, 30 mg/kg, i.p.) was administered 30 min prior to carrageenan injection (data not shown).

3.1.3. CFA-induced thermal and mechanical hyperalgesia

Following CFA injection into the hind paw, $PWL_{thermal}$ was significantly reduced (5.48±0.41 s), compared to the contralateral hind paw (10.20±0.20 s), or hind paw of saline-injected control animals (10.30±0.30 s). MPEP (3, 10, 30 mg/kg, i.p.) dose-dependently increased $PWL_{thermal}$ of CFA-injected paw, demonstrating full efficacy (93% effect) at 30 mg/kg (Fig. 3A, ED_{50}=7 mg/kg, i.p., Table 1). No effect was observed on the contralateral hind paw. In a separate group of rats, lower mechanical $PWT_{Randall}$ (175±18 g) was observed in CFA-injected paw, compared to normal paw of control rats (330±30 g). MPEP (30 mg/kg, i.p.) increased mechanical $PWT_{Randall}$ in CFA-injected paw to normal level (317±36 g) (Fig. 3B, ED_{50}=15 mg/kg, i.p., Table 1).

3.2. Effect of MPEP on visceral pain

In the mouse writhing assay, acetic acid injection induced a characteristic writhing response with 41±3 abdominal constrictions occurring during the 15 min observation period. MPEP (3, 10, 30 mg/kg, i.p.) produced a dose-dependent reduction in the number of acetic acid-induced writhes by 32%, 42%, and 81%, (P<0.05, 0.01, 0.01, respectively) (Fig. 4, ED_{50}=15 mg/kg, i.p., Table 1).

3.3. Effects of MPEP on neuropathic pain

In animals with spinal nerve ligation, a reduction in $PWT_{von\ Frey}$ was observed ipsilateral to the nerve injury (1.99±0.02 g), compared to the contralateral side (13.90±0.35 g), sham-operated animals (13.90±0.41 g) or naive animals (14.50±0.41 g). MPEP (3, 10, 30 mg/kg, i.p.) significantly increased $PWT_{von\ Frey}$ by 14%, 27%, and 33%, (P<0.05, 0.01, 0.01, respectively) (Fig. 5A, Table 1).

In animals with chronic constriction injury of the sciatic nerve, a reduction of $PWT_{von\ Frey}$ (2.45±0.18 g) was observed, compared to the contralateral side (14.04±0.32 g), and in sham-operated animals (13.60±0.72 g). MPEP (3, 10, 30 mg/kg, i.p.) also produced a moderate, but significant anti-allodynic effect in this model. At the highest dose, MPEP

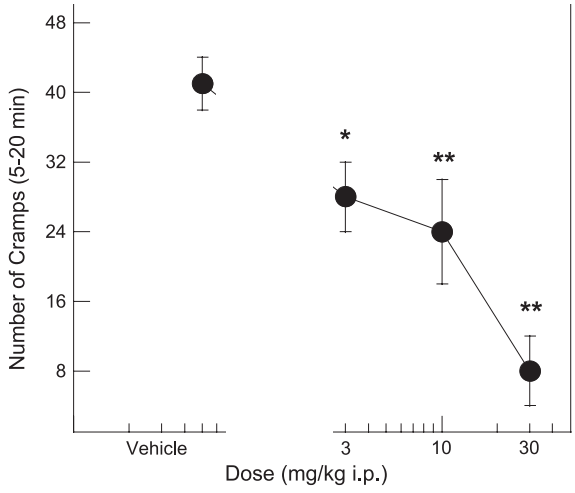

Fig. 4. MPEP dose-dependently abolished acetic acid-induced writhing activity in mice. MPEP was administered 30 min before acetic acid (0.6% in saline) injection. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group (n=8).

significantly increased $PWT_{von\ Frey}$ by 39% ($P<0.01$, Fig. 5B, Table 1).

In vincristine-treated animals, a reduction of $PWT_{von\ Frey}$ was observed (4.88±0.20 g) compared to saline-treated sham animals (12.41±0.81 g). MPEP significantly increased $PWT_{von\ Frey}$ by 40% ($P<0.01$) at 30 mg/kg (Fig. 5C, Table 1).

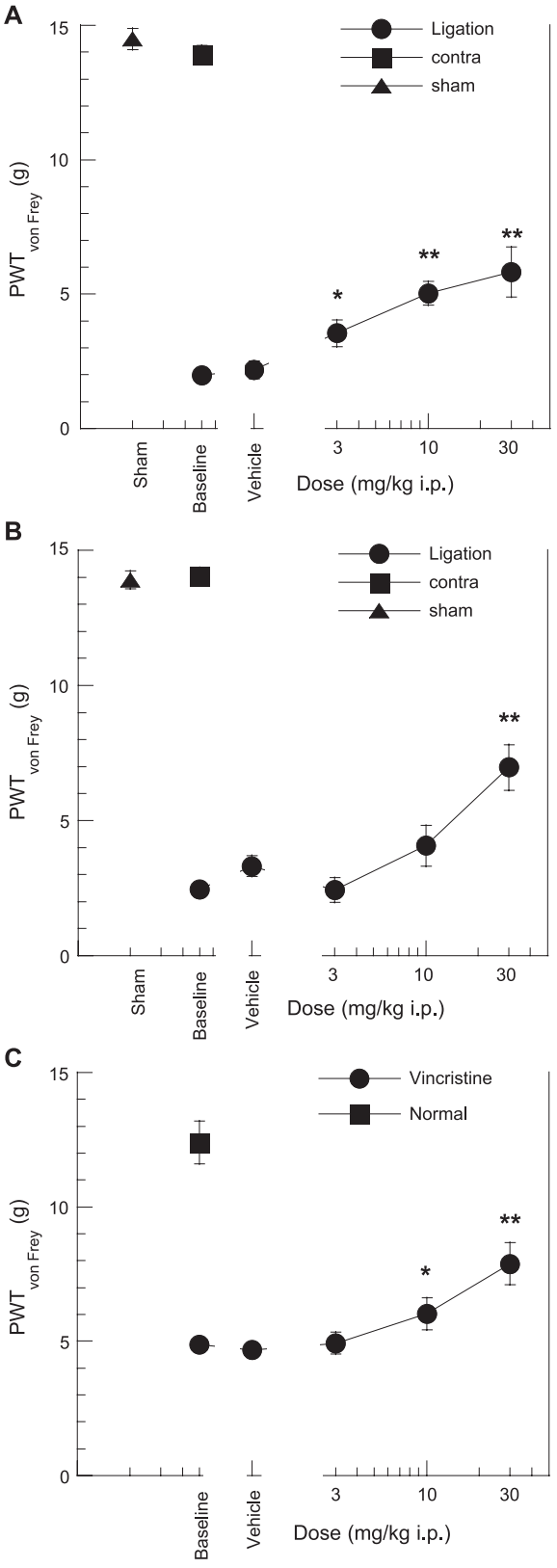

Fig. 5. Effect of MPEP on mechanical allodynia in neuropathic pain models. (A) Spinal nerve L5 and L6 ligation, (B) chronic constriction injury of the sciatic nerve and (C) vincristine-induced neuropathic pain. MPEP (30-min pretreatment) dose-dependently attenuated neuropathic pain in all three models, which is seen as an increase in the allodynic $PWT_{von\ Frey}$ of the nerve injured paw determined by using von Frey filaments. Data are mean±S.E.M. *$P<0.05$, **$P<0.01$ versus vehicle-treated group ($n=6$).

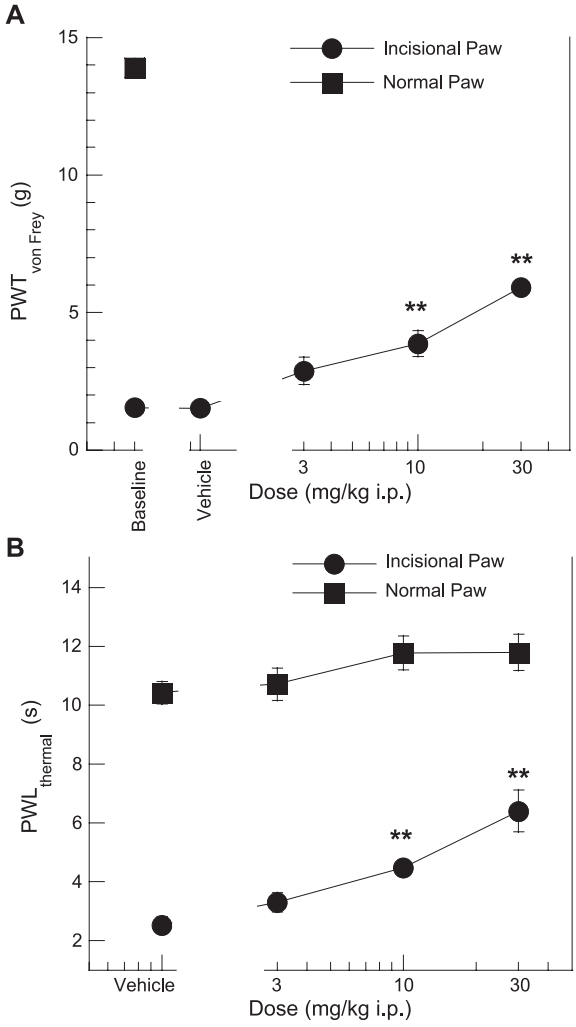

Fig. 6. MPEP significantly reduced post-operative pain as assessed by thermal hyperalgesia and mechanical allodynia in the rat 2 h post surgery. MPEP dose-dependently increased allodynic $PWT_{von\ Frey}$ (A) and $PWL_{thermal}$ (B) of the incisional paw. MPEP was administered 30 min before nociceptive behavioral assessment. Data are mean±S.E.M. *$P<0.05$, **$P<0.01$ versus vehicle-treated group ($n=6$).

3.4. Effects of MPEP on post-operative pain

Skin incision reduced $PWT_{von\ Frey}$ (1.53±0.12 g), and $PWL_{thermal}$ (2.53±0.28 s) in the injured hind paw. Skin incision did not affect either $PWT_{von\ Frey}$

(13.9±0.33 g) or $PWL_{thermal}$ (10.42±0.38 s) in the contralateral paw. MPEP (3, 10, 30 mg/kg, i.p.) dose-dependently increased $PWT_{von\ Frey}$ (Fig. 6A, Table 1), and $PWL_{thermal}$ (Fig. 6B, ED_{50}=30 mg/kg, i.p., Table 1) assessed 2 h post surgery.

3.5. Effects of MPEP on motor activity

MPEP produced no significant effects on spontaneous exploratory activity at 10 or 30 mg/kg, but significantly reduced spontaneous exploratory activity by 78% at 100 mg/kg (Fig. 7A, ED_{50}=50 mg/kg, i.p.). MPEP also produced no significant changes in fall latency on an accelerating rotating rod when compared to vehicle-treated animals at 10 or 30 mg/kg. However, a moderate, but significant effect (15%) was observed at 100 mg/kg for MPEP (Fig. 7B, ED_{50}>100 mg/kg, i.p.).

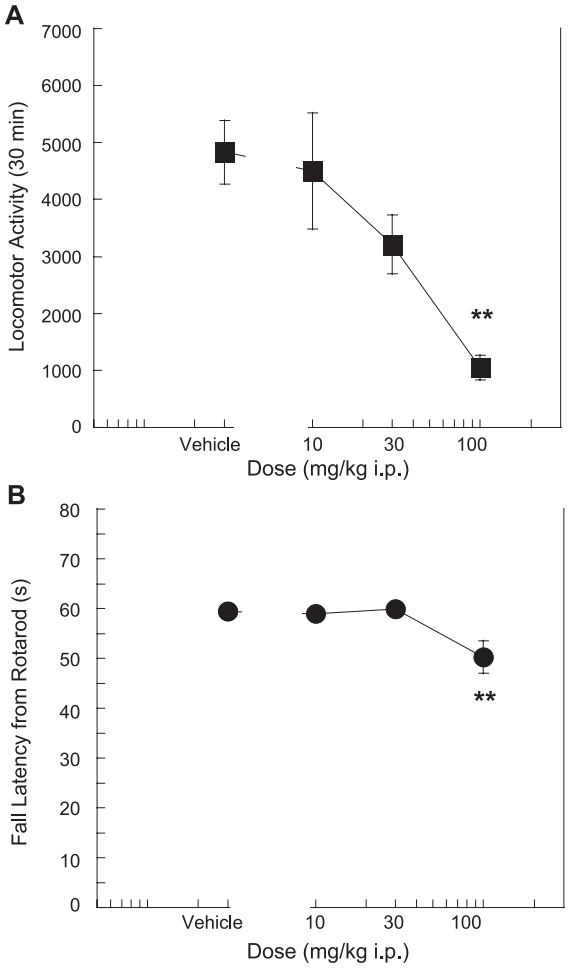

Fig. 7. Effect of MPEP on locomotor activity (A) and motor coordination measured in the rotarod assay (B) in the rat. MPEP significantly reduced locomotor activity and fall latency from the rotarod only at the highest dose administered (100 mg/kg). MPEP was administered 30 min before the assessment of motor activity. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group (n=8).

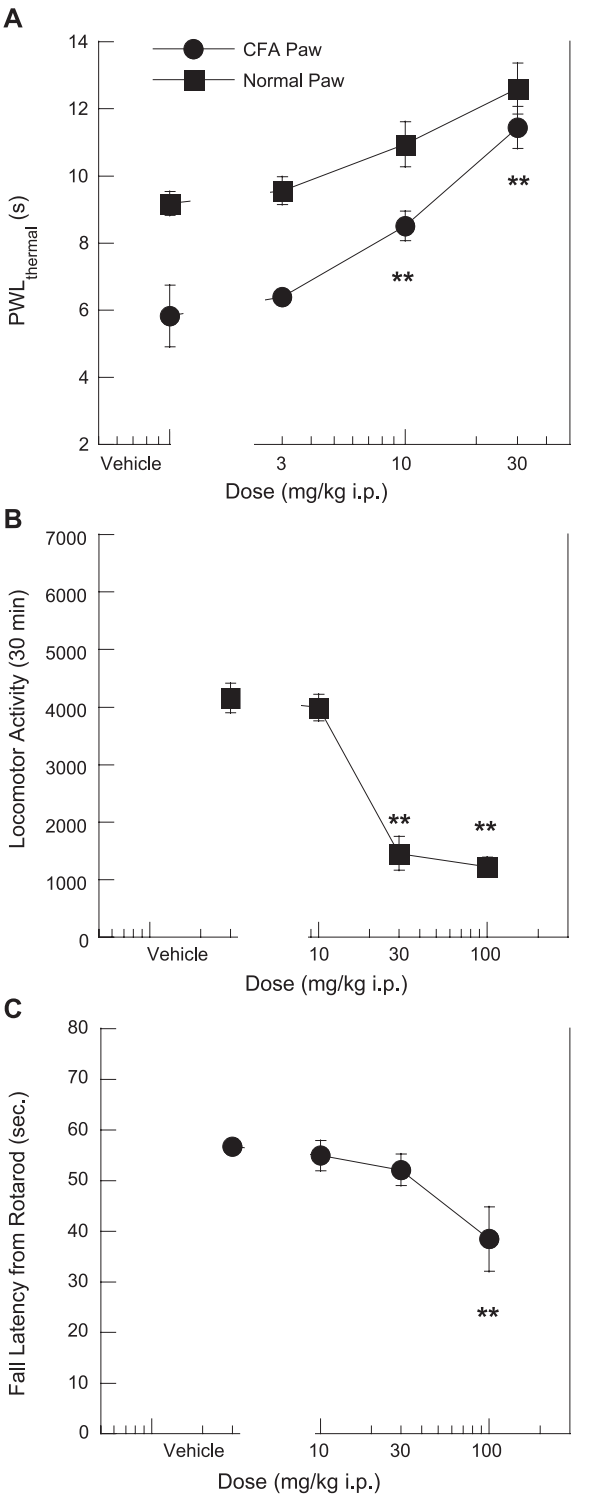

Fig. 8. Effect of MTEP on CFA-induced thermal hyperalgesia, locomotor activity and motor coordination in the rat. (A) MTEP dose-dependently increased $PWL_{thermal}$ of the CFA-injected paw to normal level, $PWL_{thermal}$ of the contralateral paw was also elevated (n=6). (B) MTEP significantly reduced locomotor activity at 30 and 100 mg/kg (n=4). (C) MTEP significantly decreased fall latency from the rotarod at the highest dose administered (100 mg/kg) (n=8). MTEP was administered 30 min before behavioral assessment. Data are mean±S.E.M. *P<0.05, **P<0.01 versus vehicle-treated group.

3.6. Effects of MTEP on selected pain and side effects models

In CFA-injected animals, MTEP dose-dependently reduced CFA-induced thermal hyperalgesia (ED_{50}=6 mg/kg, i.p.) with full (100% effect) efficacy at 30 mg/kg. However, MTEP at 10 and 30 mg/kg increased $PWL_{thermal}$ of the non-CFA injected contralateral paw to 12, and 13 s, respectively, compared to that of vehicle-treated $PWL_{thermal}$ at 9.5 s (Fig. 8A). MTEP has been shown to be as potent as MPEP on mGlu5 receptors in in vitro functional assay (Cosford et al., 2003), but given its better selectivity versus NMDA receptors, MTEP was thought to offer advantages versus MPEP in reducing poten tial locomotor side effects and decreasing potential bias in the evaluation of the role of mGlu5 receptors in nociceptive transmission. In the present study, we show that MTEP had no significant effect on spontaneous exploratory activity at 10 mg/kg, but significantly reduced spontaneous exploratory activity by 65% and 70% at 30 and 100 mg/kg, compared to vehicle-treated animals (Fig. 8B, ED_{50}=25 mg/kg, i.p.). In addition, MTEP produced no significant changes of fall latency in the rotarod assay at 10 or 30 mg/kg when compared to vehicle-treated animals. However, MTEP at 100 mg/kg significantly reduced latency to fall from the rotarod by 34% (Fig. 8C, ED_{50}>100 mg/kg, i.p.). Taken together, these data show that MTEP has as much locomotor side effects as MPEP and that the window between analgesic effects and locomotor side effects was not better than for MPEP.

4. Discussion

Findings from the present study demonstrate that i.p. administration of the potent and selective mGlu5 receptor antagonist MPEP is effective at reducing nociception, thermal hyperalgesia and mechanical allodynia in rodent models of inflammatory, visceral, neuropathic, and post-operative pain, suggesting a broad-spectrum analgesic profile for mGlu5 receptor antagonists. While MPEP was particularly potent at blocking CFA-induced thermal and mechanical hyperalgesia and acetic acid-induced visceral nociception, MPEP however, showed moderate antinociceptive activity in the skin incision model of post-operative pain, and a similar moderate effect on mechanical allodynia in three different models of neuropathic pain. In addition, MPEP was weakly active in the carrageenan and formalin assays. MTEP was reported to be as potent as MPEP in vitro but more selective for mGlu5 receptor versus NMDA receptors (Cosford et al., 2003), suggesting that it could be devoid of locomotor side effects and be more appropriate for analgesia testing. The present study is the first to examine the analgesic activity of MTEP in in vivo experiments. While MTEP was as similarly efficacious as MPEP at blocking CFA-induced thermal hyperalgesia, it also produced the same locomotor side effects and did not offer any advantages versus MPEP for analgesia testing. Therefore, it was not further examined in other nociceptive models.

mGlu5 receptor blockade was previously reported to reduce mechanical hypersensitivity in inflammatory models. For example, oral MPEP (100 mg/kg) administration produced a 70% reversal of CFA-induced mechanical hyperalgesia in response to a paw pressure, determined 24 h following CFA treatment in rats (Walker et al., 2001a). In agreement with this observation, the present study has shown that i.p. MPEP administration also dose-dependently reversed CFA-induced mechanical hyperalgesia at 48 h following CFA injection utilizing the same paw pressure test (92% reversal at 30 mg/kg, i.p.). Additionally, we have shown that i.p. MPEP or MTEP potently decreased another modality of CFA-induced hypersensitivity, thermal hyperalgesia, producing full efficacy of 93% (30 mg/kg, i.p.). I.p. MPEP administration also reduced carrageenan-induced thermal hyperalgesia without alteration of paw edema. Similarly, Walker et al. (2001a) reported that oral MPEP reversed carrageenan-induced mechanical hyperalgesia with no reduction of paw edema. In the present study, i.p. MPEP also inhibited formalin-induced phase II, but not phase I, paw flinching behaviors in rats, as was previously shown in mice following local MPEP administration (Bhave et al., 2001), suggesting that MPEP is effective at reducing inflammatory pain, rather than the neurogenic component (phase I). Taken together, these data suggest that MPEP is effective at reducing nociception as well as mechanical and thermal hypersensitivity observed in inflammatory models. The potent effects of MPEP and MTEP in the CFA model suggest that mGlu5 receptors may play a more predominant role in nociceptive transmission and modulation in chronic inflammatory pain states.

The role of mGlu5 receptors in models of neuropathic pain is controversial. For example, in a rat model of sciatic nerve partial section, oral MPEP (100 mg/kg) administration demonstrated no effect on mechanical or thermal hyperalgesia (Hudson et al., 2002; Walker et al., 2001a). In the spinal nerve ligation model, however, MPEP produced no effect on mechanical hyperalgesia or allodynia, but produced significant reduction of thermal hyperalgesia (Hudson et al., 2002). As previously shown in the spinal nerve ligation or sciatic nerve partial section models, we demonstrated that in the spinal nerve ligation, chronic constriction injury of the sciatic nerve and vincristine infusion induced neuropathic pain models, MPEP (30 mg/kg, i.p.) only partially attenuated mechanical allodynia. These results may reflect different mechanisms/involvements of mGlu5 receptor activation in thermal versus mechanical hypersensitivity in neuropathic pain states.

Regardless of modality of testing, mGlu5 receptors seem to play a more prominent role in inflammatory

versus neuropathic pain states. Several lines of evidence could reasonably explain this difference. It has been shown that mGlu5 receptors are widely expressed on C-fiber afferent terminals that are primarily mediating thermal nociceptive transmission (Ossipov et al., 1999), and mGlu5 receptors are more sparsely expressed on Aβ afferent fibers which are believed to play a major role in mechanical allodynia (Bhave et al., 2001; Walker et al., 2001b). Co-localization of both mGlu5 receptors and vanilloid receptors on C-fibers provides the foundation for the potential functional coupling of mGlu5 and vanilloid receptors (Walker et al., 2001b), supporting the fact that the interaction between vanilloid and mGlu5 receptors may contribute to the inhibition of thermal hyperalgesia in inflammatory pain. However, mGlu5 receptor expression has also been shown to be increased in ipsilateral L4 and L5 Aβ fibers following spinal nerve ligation or sciatic nerve injury (Hudson et al., 2002), and this increased expression is in agreement with the modest efficacy observed in three models of neuropathic pain in the present study. Furthermore, mGlu5 receptors are localized post-synaptically on second order neurons (Jia et al., 1999), and mechanical allodynia may also be attenuated as a consequence of diminished central sensitization mediated by post-synaptic mGlu5 receptors. The potential interaction of MPEP with other types of glutamate receptors to produce nociceptive effects should also be considered, although this compound had no significant agonist or antagonist activity at any other mGlu or iGlu receptor subtypes up to 100 μM (Gasparini et al., 1999a). Local injection of iGlu receptor, mGlu receptor group II, or group III antagonists showed no effect on DHPG-induced thermal hyperalgesia (Bhave et al., 2001). However, previous studies involving peripheral injection of excitatory amino acids have also found that iGlu receptor antagonists completely eliminate glutamate-induced hypersensitivity (Carlton and Coggeshall, 1999; Lawand et al., 2000). Therefore, it may be a general phenomenon of peripheral glutamatergic signaling that activation of iGlu and mGlu5 receptors can lead to increased thermal sensitivity, whereas blockade of any one of these receptor subtypes can prevent the effects of glutamate. MPEP seems to decrease the overall glutamatergic neurotransmission, consequently leading to an overall inhibitory effect in both peripheral and central nociceptive sensitization, thereby exerting its antinociceptive activity.

The potential antinociceptive activity of mGlu5 receptor blockade on visceral pain is less well explored; i.p. administration of a nonselective group I mGlu receptor antagonist, LY393053 ((±)-2-amino-2-(3-*cis* and *trans*-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid), produced a dose-dependent reduction in acetic acid-induced writhing activity in mice at doses 1–10 mg/kg, with ED_{50} value of 6.0 mg/kg (Chen et al., 2000). In the present study, i.p. MPEP administration fully blocked acetic acid-induced writhing activity (ED_{50}=15 mg/kg, i.p.). In contrast, i.t. MPEP administration did not suppress visceral pain in a pancreatitis model (Zhang and Westlund, 2002).

The present study is the first to investigate the role of mGlu5 receptors in post-operative pain using a highly potent and selective compound. Previously, it was shown that mechanical allodynia was not relieved by i.t. administration of nonselective group I mGlu receptor antagonists in rat skin incision model (Zahn and Brennan, 1998a). However, in the present study i.p. MPEP partially attenuated acute thermal hyperalgesia, and mechanical allodynia observed 2 h post surgery. Interestingly, MPEP was efficacious in the acute phase of this model of post-operative pain, in which very few analgesics have been demonstrated to be efficacious. For example, while both spinal and systemic morphine have shown similar analgesic activity in acute post-operative pain behaviors and other modalities of nociception (Zahn et al., 1997), i.t. NMDA receptor antagonists do not produce any analgesic effects on pain behaviors following plantar incision (Zahn and Brennan, 1998b). Likewise, i.t. cyclo-oxygenase-2 inhibitors attenuate inflammation-induced mechanical allodynia and thermal hyperalgesia, while they do not exert any effects on mechanical hypersensitivity following paw incision (Kroin et al., 2002; Zhu et al., 2003).

The present study showed that i.p. MPEP had no effects on motor coordination, as reflected by rotarod latency, at doses providing threshold antinociceptive activity in all animal models studied. However, i.p. MPEP at high dose (100 mg/kg) affected motor coordination and exploratory behavior; therefore, higher doses of MPEP were not tested in this study to remove any confounding factor from the evaluation of MPEP antinociceptive activity.

In conclusion, in the present study, we demonstrated, for the first time, that MTEP, a recently described potent and selective mGlu5 receptor antagonist produces analgesic efficacy in vivo. In addition, whereas MTEP is more selective than MPEP versus NMDA receptors, MTEP in vivo efficacy is associated with locomotor side effects suggesting that MTEP does not offer any advantages versus MPEP for characterizing the potential analgesic profile of mGlu5 receptor blockade. Furthermore, in addition to confirming the potent effects of MPEP on inflammatory mechanical hyperalgesia and weak effects on mechanical allodynia observed in models of spinal nerve ligation and chronic constriction injury of the sciatic nerve, we demonstrate for the first time that MPEP potently inhibits CFA-induced inflammatory thermal hyperalgesia and visceral pain, in addition to having moderate but significant effects in post-operative pain, and chemotherapy-induced neuropathic pain. The present data, along with previous reports, demonstrate that mGlu5 receptors play a role in multiple nociceptive modalities and in multiple pain states suggesting a potential broad-

spectrum analgesia for mGlu5 receptor antagonists, though CNS side effects may be a limiting factor in developing mGlu5 receptor analgesic agents.

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References

Abbott, F.V., Franklin, K.B., Westbrook, R.F., 1995. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 60, 91–102.

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

Bhave, G., Karim, F., Carlton, S.M., Gereau, R.W., 2001. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat. Neurosci. 4, 417–423.

Bordi, F., Ugolini, A., 1999. Group I metabotropic glutamate receptors: implications for brain diseases. Prog. Neurobiol. 59, 55–79.

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F., 1996. Characterization of a rat model of incisional pain. Pain 64, 493–501.

Carlton, S.M., Coggeshall, R.E., 1999. Inflammation-induced changes in peripheral glutamate receptor populations. Brain Res. 820, 63–70.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53, 55–63.

Chen, Y., Bacon, G., Sher, E., Clark, B.P., Kallman, M.J., Wright, R.A., Johnson, B.G., Schoepp, D.D., Kingston, A.E., 2000. Evaluation of the activity of a novel metabotropic glutamate receptor antagonist (±)-2-amino-2-(3-cis and *trans*-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid) in the in vitro neonatal spinal cord and in an in vivo pain model. Neuroscience 95, 787–793.

Cosford, N.D., Tehrani, L., Roppe, J., Schweiger, E., Smith, N.D., Anderson, J., Bristow, L., Brodkin, J., Jiang, X., McDonald, I., Rao, S., Washburn, M., Varney, M.A., 2003. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. J. Med. Chem. 46, 204–206.

Crawford, J.H., Wainwright, A., Heavens, R., Pollock, J., Martin, D.J., Scott, R.H., Seabrook, G.R., 2000. Mobilisation of intracellular Ca^{2+} by mGluR5 metabotropic glutamate receptor activation in neonatal rat cultured dorsal root ganglia neurones. Neuropharmacology 39, 621–630.

Dixon, W.J., 1980. Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. 20, 441–462.

Dogrul, A., Ossipov, M.H., Lai, J., Malan Jr., T.P., Porreca, F., 2000. Peripheral and spinal antihyperalgesic activity of SIB-1757, a metabotropic glutamate receptor (mGLUR(5)) antagonist, in experimental neuropathic pain in rats. Neurosci. Lett. 292, 115–118.

Dolan, S., Nolan, A.M., 2000. Behavioural evidence supporting a differential role for group I and II metabotropic glutamate receptors in spinal nociceptive transmission. Neuropharmacology 39, 1132–1138.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174.

Fisher, K., Coderre, T.J., 1998. Hyperalgesia and allodynia induced by intrathecal (RS)-dihydroxyphenylglycine in rats. NeuroReport 9, 1169–1172.

Fisher, K., Fundytus, M.E., Cahill, C.M., Coderre, T.J., 1998. Intrathecal administration of the mGluR compound, (S)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats. Pain 77, 59–66.

Fundytus, M.E., Fisher, K., Dray, A., Henry, J.L., Coderre, T.J., 1998. In vivo antinociceptive activity of anti-rat mGluR1 and mGluR5 antibodies in rats. NeuroReport 9, 731–735.

Gasparini, F., Bruno, V., Battaglia, G., Lukic, S., Leonhardt, T., Inderbitzin, W., Laurie, D., Sommer, B., Varney, M.A., Hess, S.D., Johnson, E.C., Kuhn, R., Urwyler, S., Sauer, D., Portet, C., Schmutz, M., Nicoletti, F., Flor, P.J., 1999a. (R,S)-4-phosphonophenylglycine, a potent and selective group III metabotropic glutamate receptor agonist, is anticonvulsive and neuroprotective in vivo. J. Pharmacol. Exp. Ther. 289, 1678–1687.

Gasparini, F., Lingenhohl, K., Stoehr, N., Flor, P.J., Heinrich, M., Vranesic, I., Biollaz, M., Allgeier, H., Heckendorn, R., Urwyler, S., Varney, M.A., Johnson, E.C., Hess, S.D., Rao, S.P., Sacaan, A.I., Santori, E.M., Velicelebi, G., Kuhn, R., 1999b. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38, 1493–1503.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J., 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77–88.

Hudson, L.J., Bevan, S., McNair, K., Gentry, C., Fox, A., Kuhn, R., Winter, J., 2002. Metabotropic glutamate receptor 5 upregulation in A-fibers after spinal nerve injury: 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reverses the induced thermal hyperalgesia. J. Neurosci. 22, 2660–2668.

Jia, H., Rustioni, A., Valtschanoff, J.G., 1999. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. J. Comp. Neurol. 410, 627–642.

Kim, S.H., Chung, J.M., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355–363.

Kroin, J.S., Buvanendran, A., McCarthy, R.J., Hemmati, H., Tuman, K.J., 2002. Cyclooxygenase-2 inhibition potentiates morphine antinociception at the spinal level in a postoperative pain model. Reg. Anesth. Pain Med. 27, 451–455.

Lawand, N.B., McNearney, T., Westlund, K.N., 2000. Amino acid release into the knee joint: key role in nociception and inflammation. Pain 86, 69–74.

Lorrain, D.S., Correa, L., Anderson, J., Varney, M., 2002. Activation of spinal group I metabotropic glutamate receptors in rats evokes local glutamate release and spontaneous nociceptive behaviors: effects of 2-methyl-6-(phenylethynyl)-pyridine pretreatment. Neurosci. Lett. 327, 198–202.

Mogil, J.S., Wilson, S.G., Bon, K., Lee, S.E., Chung, K., Raber, P., Pieper, J.O., Hain, H.S., Belknap, J.K., Hubert, L., Elmer, G.I., Chung, J.M., Devor, M., 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80, 67–82.

Neugebauer, V., 2002. Metabotropic glutamate receptors—important modulators of nociception and pain behavior. Pain 98, 1–8.

Nozaki-Taguchi, N., Chaplan, S.R., Higuera, E.S., Ajakwe, R.C., Yaksh, T.L., 2001. Vincristine-induced allodynia in the rat. Pain 93, 69–76.

Omote, K., Kawamata, T., Kawamata, M., Namiki, A., 1998. Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res. 787, 161–164.

Ossipov, M.H., Bian, D., Malan Jr., T.P., Lai, J., Porreca, F., 1999. Lack of involvement of capsaicin-sensitive primary afferents in nerve-ligation injury induced tactile allodynia in rats. Pain 79, 127–133.

Salt, T.E., Binns, K.E., Turner, J.P., Gasparini, F., Kuhn, R., 1999. Antagonism of the mGlu5 agonist 2-chloro-5-hydroxyphenylglycine by the novel selective mGlu5 antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) in the thalamus. Br. J. Pharmacol. 127, 1057–1059.

Stein, C., Millan, M.J., Herz, A., 1988. Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. Pharmacol. Biochem. Behav. 31, 451–455.

Varney, M.A., Cosford, N.D., Jachec, C., Rao, S.P., Sacaan, A., Lin, F.F., Bleicher, L., Santori, E.M., Flor, P.J., Allgeier, H., Gasparini, F., Kuhn, R., Hess, S.D., Velicelebi, G., Johnson, E.C., 1999. SIB-1757 and SIB-1893: selective, noncompetitive antagonists of metabotropic glutamate receptor type 5. J. Pharmacol. Exp. Ther. 290, 170–181.

Walker, K., Bowes, M., Panesar, M., Davis, A., Gentry, C., Kesingland, A., Gasparini, F., Spooren, W., Stoehr, N., Pagano, A., Flor, P.J., Vranesic, I., Lingenhoehl, K., Johnson, E.C., Varney, M., Urban, L., Kuhn, R., 2001. Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function: I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. Neuropharmacology 40, 1–9.

Walker, K., Reeve, A., Bowes, M., Winter, J., Wotherspoon, G., Davis, A., Schmid, P., Gasparini, F., Kuhn, R., Urban, L., 2001. mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. Neuropharmacology 40, 10–19.

Zahn, P.K., Brennan, T.J., 1998a. Intrathecal metabotropic glutamate receptor antagonists do not decrease mechanical hyperalgesia in a rat model of postoperative pain. Anesth. Analg. 87, 1354–1359.

Zahn, P.K., Brennan, T.J., 1998b. Lack of effect of intrathecally administered *N*-methyl-D-aspartate receptor antagonists in a rat model for postoperative pain. Anesthesiology 88, 143–156.

Zahn, P.K., Gysbers, D., Brennan, T.J., 1997. Effect of systemic and intrathecal morphine in a rat model of postoperative pain. Anesthesiology 86, 1066–1077.

Zahn, P.K., Sluka, K.A., Brennan, T.J., 2002. Excitatory amino acid release in the spinal cord caused by plantar incision in the rat. Pain 100, 65–76.

Zhang, L.P., Westlund, K.N., 2002. Restoration of spontaneous exploratory behaviors by mGlu R1 antagonists in rats with pancreatitis. J. Neurosci., 451.11.

Zhu, X., Conklin, D., Eisenach, J.C., 2003. Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. Pain 104, 15–23.