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ORIGINAL INVESTIGATION

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The antinociceptive and anxiolytic-like effects of the metabotropic glutamate receptor 5 (mGluR5) antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in rodents: a comparison of efficacy and side-effect profiles

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Abstract *Rationale:* Modulation of metabotropic glutamate receptor (mGluR) subtypes represents a novel approach for the treatment of neurological and psychiatric disorders. *Objectives:* This study was conducted to investigate the role of the mGluR5 and mGluR1 subtypes in the modulation of pain and anxiety. *Methods:* The mGluR5 antagonists, 2-methyl-6-(phenylethynyl)pyridine (MPEP) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), and the mGluR1 antagonist, (4-methoxy-phenyl)-(6-methoxy-quinazolin-4-yl)-amine HCl (LY456236), were tested in models of pain [mouse formalin test, rat spinal nerve ligation (SNL)] and anxiety [Vogel conflict, conditioned lick suppression (CLS)], and their efficacious effects were compared to any associated side effects. *Results:* The systemic administration of MPEP, MTEP, and LY456236 reduced hyperalgesia induced by formalin and mechanical allodynia following SNL. However, only LY456236 completely reversed the allodynia. In the anxiety models, MPEP (3–30 mg/kg), MTEP (3–10 mg/kg), and LY456236 (10–30 mg/kg) produced anxiolytic-like effects similar to the benzodiazepine, chlordiazepoxide (CDP, 6 mg/kg). However, only MPEP and MTEP were able to produce a level of anxiolysis comparable to CDP. In a series of tests examining potential side effects, MPEP and MTEP reduced body temperature and locomotor activity and impaired operant responding for food and rotarod performance at doses of 3–30 and 1–30 mg/kg, respectively. LY456236 reduced operant responding at 30 mg/kg. *Conclusion:* Both mGluR5 and mGluR1 antagonists are effective in models of pain and anxiety. However, an mGluR1 antagonist was more efficacious than the two mGluR5 antagonists in the pain models, which, conversely, appeared more efficacious in the anxiety models. These findings support the potential utility of mGluR5 and mGluR1 antagonists for both the treatment of chronic pain and as novel anxiolytics.

Keywords mGluR1 · mGluR5 · Neuropathic pain · Anxiolysis · Behavior

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Introduction

The ability of glutamate to act on a large number of receptors provides opportunities to modulate the activity of glutamatergic synapses and to potentially treat disorders that involve alterations in glutamatergic neurotransmission. Glutamate synaptic responses in the central nervous system (CNS) are mediated via the activation of two families of receptors: ligand-gated cation channels termed *ionotropic glutamate receptors* and G-protein-coupled receptors known as *metabotropic glutamate receptors* or *mGluRs*. Thus far, eight mGluR subtypes, together with splice variants, have been cloned and characterized in functional studies (Schoepp et al. 1999). The eight mGluRs are grouped into three classes based on structural homology, pharmacology, and signal transduction mechanisms: group I receptors (mGluRs 1 and 5) are coupled to phospholipase

C and stimulate phosphoinositide hydrolysis and intracellular Ca^{2+} signal transduction, whereas group II and group III receptors are negatively coupled to adenyl cyclase (Nakanishi 1994; Conn and Pin 1997). Among these receptors, the recent identification of noncompetitive group I subtype-specific antagonists has greatly accelerated the investigation and the elucidation of the role of mGluR1 and mGluR5 in animal models of neurological and psychiatric disorders.

A number of behavioral (Fisher and Coderre 1998; Fundytus et al. 1998; Bhave et al. 2001; Dolan and Nolan 2002; Dolan et al. 2003) and electrophysiological (Young et al. 1994, 1997) studies have demonstrated a specific role for group I mGluRs in nociceptive processing in the CNS, including mechanisms of hyperalgesia and inflammation. mGluR1 appears to be localized primarily on postsynaptic elements throughout the dorsal and ventral horns of the spinal cord (Neugebauer 2001). Behavioral studies have demonstrated that intrathecal administration of the mGluR1 antagonist, CPCCOEt, produces antinociceptive effects in the second phase of formalin-induced nociceptive behavior (Neugebauer 2001). Additionally, expression of mGluR1 is increased in rats following spinal cord injury, and this may mediate the chronic central pain induced by the injury (Mills and Hulsebosch 2002). Knockdown of spinal mGluR1 by intrathecal infusion of antisense oligonucleotides attenuated cold hyperalgesia and mechanical allodynia in neuropathic rats (Fundytus et al. 2001, 2002). Additionally, spinal administration of anti-mGluR1 IgG antibodies reduced cold hyperalgesia, but not mechanical allodynia, in neuropathic rats (Fundytus et al. 1998). The critical role of spinal mGluR1 receptors in pain-related central sensitization is emphasized at the single cell level by electrophysiological in vivo studies in anesthetized animals. Intraspinal administration of the mGluR1 antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA), inhibited the responses of primate spinothalamic tract neurons to brief, noxious, but not innocuous, mechanical cutaneous stimuli, as well as central sensitization in the capsaicin pain model (Neugebauer et al. 1999). In rats with knocked down mGluR1 expression, the responses of multireceptive dorsal horn neurons to noxious input evoked by repeated topical applications of the C-fiber irritant mustard oil were significantly reduced compared to control neurons; the responses to innocuous cutaneous stimuli were unaffected (Young et al. 1998).

mGluR5 receptors are expressed both pre- and postsynaptically in the superficial dorsal horn on the spinal cord (Neugebauer 2001). Similar to mGluR1, the intrathecal administration of the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) produced antinociceptive effects in the second phase of the formalin model (Karim et al. 2001), and the spinal administration of anti-mGluR5 IgG antibodies reduced cold hyperalgesia in neuropathic rats (Fundytus et al. 1998). Recent studies also demonstrated the attenuating effects of systemically administered MPEP on nociceptive behavior in neuropathic pain models (Walker et al. 2001a,b; Fisher et al. 2002; Hudson et al. 2002). Finally, electrophysiological recordings in a hemisected spinal cord in vitro preparation suggest that mGluR5 receptors may be involved in brief and prolonged spinal nociception (Bordi and Ugolini 2000). Clearly, the mGluR5 receptor subtype has a role in the modulation of pain, but the current evidence is perhaps not as strong as for mGluR1.

The predominant expression of mGluR5 in areas of the mammalian brain thought to be involved in emotional processes has suggested a possible role in affective disorders, especially stress and anxiety (see Bordi and Ugolini 1999 for review). Several reports describing the effects of mGluR5 antagonists in various animal models provide compelling evidence for a broad anxiolytic-like activity of mGluR5 antagonists (Klodzinska et al. 2000; Spooren et al. 2000; Schulz et al. 2001; Tatarczynska et al. 2001; Brodkin et al. 2002a). The anxiolytic-like effects of mGluR5 antagonists, particularly MPEP (Gasparini et al. 1999), have been demonstrated in both rats and mice across a range of commonly used models. Specifically, mGluR5 antagonists produce anxiolytic-like effects in unconditioned response models, including the elevated plus maze, stress-induced hyperthermia, and Vogel conflict tests, and conditioned response models, such as fear-potentiated startle, a conditioned ultrasonic vocalization (USV) procedure, and the Geller–Seifter test (Klodzinska et al. 2000; Spooren et al. 2000; Schulz et al. 2001; Tatarczynska et al. 2001; Brodkin et al. 2002a). Encouragingly, the anxiolytic-like effects of mGluR5 antagonists appear to be in the magnitude of the benzodiazepine anxiolytics, but with an improved side-effect profile. Additionally, in support of the role of mGluR5 in stress and anxiety, Brodkin et al. (2002b) demonstrated that the mGluR5 knockout mouse had a reduced hyperthermic response to stressful stimuli. Unlike mGluR5, very little is known about the anxiolytic potential of mGluR1 antagonists, although a recent report from Klodzinska et al. (2004a) demonstrated the anxiolytic-like effects of the selective mGluR1 antagonist, AIDA, and a reduced propensity to induce the side effects characteristic of benzodiazepines.

Given this evidence, and, as to our knowledge, no studies have directly compared the profile of mGluR5 and mGluR1 antagonists in pain and anxiety, the aim of this study was to test the available pharmacological tools in representative animal models. Specifically, the noncompetitive mGluR5 antagonists 2-methyl-6-(phenylethynyl)pyridine (MPEP; Gasparini et al. 1999) and 3-[(2-methyl-1,3-thiazol-4-yl) ethynyl]pyridine (MTEP; Anderson et al. 2002), and the noncompetitive mGluR1 antagonist, (4-methoxy-phenyl)-(6-methoxy-quinazolin-4-yl)-amine HCl (LY456236; Barton et al. 2003), were used. Although a significant number of studies have used MPEP as a standard antagonist (Karim et al. 2001; Walker et al. 2001a,b; Fisher et al. 2002; Hudson et al. 2002), to date, no work has been published on MTEP or LY456236. From the available literature, the reported affinities (K_i values) of MPEP and MTEP at rat mGluR5 receptors are 12 and 16 nM, respectively (Cosford et al. 2003). From our in vitro studies, LY456236 had an in vitro affinity of 143 nM at rat mGluR1 receptors (measured by

displacement of [^{3}H]R214127 binding in rat membranes as described by Lavreysen et al. 2003). Compound selectivity within the mGluR family was also confirmed using assays employing nonneuronal cell lines (HEK293) stably expressing recombinant human mGluR1a, mGluR5b, mGluR2, and mGluR8. Up to concentrations of 10 mM, MPEP and MTEP were devoid of activity at human mGluR1a, mGluR2, and mGluR8a, and LY456236 had no effect on human mGluR5b, mGluR2, and mGluR8a (measured by Ca^{2+} flux assay). In these studies, MPEP, MTEP, and LY456236 were tested in a number of established rodent models of pain, as well as models sensitive to anxiolytic drugs. Although a number of publications have described the effects of mGluR1 or mGluR5 antagonists in models of pain or models sensitive to anxiolytic drugs (Neugebauer et al. 1999; Bordi and Ugolini 2000; Dogrul et al. 2000; Klodzinska et al. 2000, 2004a; Spooren et al. 2000; Neugebauer 2001), to our knowledge, this is the first study to directly compare these classes of mGluR antagonists in both sets of assays. Additionally, MPEP, MTEP, and LY456236 were tested in a set of behavioral assays that assessed neurological function (motor coordination, body temperature, response rate) in order to evaluate the efficacy and side-effect profiles of these mGluR antagonists, which, again, have not been directly compared in the same study.

Materials and methods

Subjects

Male CD1 mice weighing 25–30 g and male CD rats weighing 150–175 g (Charles River, Calco, Italy) were used in the formalin and spinal nerve ligation (SNL) experiments, respectively. Male CD rats (Charles River, Kingston, NY, USA) weighing 225–250 g were used for the remaining studies. Mice were housed ten per cage, whereas rats were housed singly [conditioned lick suppression (CLS) and fixed-ratio (FR) studies—see below] or three per cage (all other studies), under a 12 h light–dark cycle (lights on 0700 h) with constant temperature and humidity. Throughout the studies, animals were given free access to food (except during the FR studies) and water (except during Vogel and CLS studies). Rats were used once only, i.e., for a single administration of drug or vehicle (except in the SNL, CLS and FR assays—see below). All studies took place during the light cycle between 0800 and 1700 h. All surgical and testing procedures involving animals and their care were conducted in conformity with the institutional guidelines and in compliance with the European Community Council Directive 86/609 (OJ L 358, 1, December 12, 1987), the NIH's *Guide to the Care and Use of Laboratory Animals*, and the Animal Welfare Act.

Mouse formalin test

Mice were gently restrained and 30 μl of formalin solution (1.5% in saline) was injected subcutaneously into the plantar surface of the right hind paw of the mouse, using a microsyringe with a 27-gauge needle. After the formalin injection, the mouse was immediately placed into a Plexiglas observation box (30×20×20 cm), and the nociceptive response of the mouse to the formalin injection was observed for a period of 60 min. The duration of licking and flinching of the injected paw was recorded and quantified every 5 min for the total observation period. The recording of the early phase (first phase) started immediately and lasted for 5 min. The late phase (second phase) started 10–15 min after formalin injection. The period 15–30 min after formalin injection was used for pharmacological studies since this period incorporates the peak formalin effect.

L5 and L6 spinal nerve ligation of the rat sciatic nerve

Peripheral neuropathy was produced by ligating the L5 and L6 spinal nerves of the right sciatic nerve, according to the method previously described by Kim and Chung (1992). Briefly, rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a prone position, and the right paraspinal muscles were separated from the spinal processes at the L4–S2 levels. The L5 transverse process was carefully removed with a small rongeur to identify the L4–L5 spinal nerves. The right L5 and L6 spinal nerves were isolated and tightly ligated with 7–0 silk thread. A complete hemostasis was confirmed and the wound was sutured.

Measurement of tactile allodynia

Tactile sensitivity was evaluated using a series of calibrated Semmes-Weinstein (Stoelting, IL) von Frey filaments, with a bending force ranging from 0.25 to 15 g. Rats were placed in a transparent plastic box endowed with a metal mesh floor and were habituated to this environment prior to testing. The von Frey filaments were applied perpendicularly to the midplantar surface of the ipsilateral hind paws, and the mechanical allodynia was determined by sequentially increasing and decreasing the stimulus strength ("up–down" procedure of filament presentation). The 50% paw withdrawal threshold was determined by the nonparametric Dixon test (Chaplan et al. 1994). Paw licking and vigorously shaking after stimulation were considered pain-like responses. Only those rats that demonstrated a threshold less than 4 g (commonly considered in the literature as the tactile allodynia threshold) on postoperative day 14 were included in the behavioral studies (more than 90% of the animals). Compounds were tested using within-subjects studies such that each rat received vehicle and all doses of a compound in a random, crossover design.

Vogel conflict

Testing took place in eight standard operant conditioning boxes (32×25×25 cm) (Med Associates, Camden, VT). Each box contained a stainless-steel drinking spout that protruded through the center of the front wall 3 cm above the floor. The drinking spout was connected to a 200-ml bottle containing 0.2% saccharin solution. Each box was located within a ventilated and sound-attenuated chamber containing a 5-W house light. Licks were recorded automatically by a lickometer connected to a computer. Scrambled foot shock (0.4 mA, 500-ms duration) was delivered to the spout and grid floor of the box upon completion of every 20th lick. Prior to any testing in the boxes, rats were deprived of water for ~20 h. Additionally, the day before drug testing, rats were placed into the boxes and allowed free, unpunished access to the saccharin solution for 10 min to establish a robust baseline level of licking. On the test day, rats were brought to the test room and allowed to acclimate for 1 h. Drug or vehicle was administered, and, following an appropriate pretreatment time, rats were placed into the boxes and the numbers of licks during a 10 min test session were recorded.

Conditioned lick suppression

Experiments took place in the Vogel conflict apparatus. A cohort of 60 rats was water-deprived for 20 h and trained under a schedule of CLS. Briefly, rats were presented with 20 trials, each consisting of 23 s of unpunished drinking, followed by 7 s in which a tone was delivered. Licks during the first 2 s of tone were unpunished, whereas licks during the final 5 s were punished with scrambled shock delivery (0.7 mA, 500-ms duration). Rats were trained under this schedule 6 days per week until they made fewer than 5% of total licks during the period in which tone was presented. When rats had reached this criterion, they were tested during a session in which tone was presented but shock delivery was turned off. If rats made less than 5% of total licks during the tone period under these conditions, they were deemed to be conditioned and suitable for drug testing. On the day of testing, rats were brought to the test room and administered vehicle or a dose of drug. After an appropriate pretreatment, rats were placed into the apparatus, and the number of licks during both the unpunished (no tone) and punished (tone, no shock) components of the test was recorded for 20 trial presentations. The drug was administered using a within-subjects, crossover design such that the effect of vehicle administration and up to five doses of drug [including a maximal effective 6 mg/kg dose of the positive control, chlordiazepoxide (CDP)] was assessed over several different test sessions. The drugs were tested no more than twice a week. On intervening days, rats continued to be trained in sessions with both tone and concomitant shock. Rats received one or two of the drugs tested in these studies with sufficient washout between testing.

Body temperature

A rectal probe and a digital thermometer (Physitemp, Clifton, NJ) were used to measure core body temperature in rats. The probe was lubricated with mineral oil and inserted into the rectum. Body temperature was recorded prior to injection (mean baseline temperature: MPEP=36.8°C; MTEP=36.8°C; LY456236=37.3°C) and every 30 min following injection for 3 h. For brevity, only data from the 2 h reading (the time when the maximum effect was observed) were analyzed for each drug.

Locomotor activity

Rats were transferred from their home cages to an anteroom in which they were allowed to habituate for 1 h. Following dosing and the appropriate pretreatment time, activity levels were recorded for 2 h using a Versamax LMA system (Accuscan Instruments, Columbus, OH). Each monitoring system consisted of a Plexiglas box (height 30 cm, width 42 cm, length 42 cm), and activity was monitored by *xy*-axis photobeams located 2 cm above the floor and spaced 2.5 cm apart, which sampled 100 times per second. Data were transferred to a computer that digitized and stored the data for separate analysis.

Rotarod

Rats were pretrained to a performance criterion on a rotarod (Ugo Basile: rod width 8.5 cm, diameter 7 cm) 24 h prior to testing. Rats were required to remain on the rod rotating at 16 rpm for 120 s during two successive trials (>95% of animals attained this criterion). On test day, rats were tested in six trials, three each at 8 and 16 rpm. The duration on the rod was recorded up to a maximum of 120 s, and only the best trial at each speed was used in the final analysis.

Beam walking

Rats were pretrained to a performance criterion of successfully traversing the beam (length 90 cm, width 2 cm, elevation 43 cm) in order to enter a dark box within 60 s without any foot slips, twice during both morning and afternoon training sessions (>95% of animals attained this criterion). On test day, each rat was given two trials on the beam. The distance traversed along the beam was recorded, and the best performance out of the two trials was recorded and used in the final analysis.

Fixed-ratio responding

Studies were conducted in 12 operant conditioning boxes housed in sound-attenuating chambers (MED Associates, Georgia, VT). Rats were trained to lever press for 45 mg food pellets (Bio-Serv, Frenchtown, NJ). To maintain

motivation, rats were kept at 80% of their normal free-feeding body weight. Training began at an FR schedule of reinforcement of 1 (FR1, i.e., one lever press is required to obtain one food pellet), and the FR was gradually increased to FR10 (ten lever presses per pellet). Sessions lasted either 60 min or until a rat had received 100 pellets, whichever occurred first. Specially designed software controlled the boxes, ran the FR program, and collected the data (KESTREL system, Conclusive Solutions, Cambridge, UK). Drugs were tested in a within-subjects, crossover-designed study such that each rat served as its own control. Rats received one or two of the drugs tested in these studies with sufficient washout between testing.

Drugs

Formalin was prepared by diluting a formaldehyde solution (37%, Scharlau, Barcelona, Spain) with saline to a final concentration of 1.5%. MPEP hydrochloride, MTEP hydrochloride, and LY456236 were synthesized by the Medicinal Chemistry Department of the Schering Plough Research Institute. Chlordiazepoxide hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). MPEP, MTEP, and LY456236 were dissolved in a 10% Tween 80/90% distilled water vehicle, and CDP was dissolved in 0.9% saline. All drugs were administered by the intraperitoneal (i.p.) route at volumes of 1 ml/kg (rat) or 10 ml/kg (mouse), and all doses are expressed as free base.

Statistical analysis

Data from the mouse formalin test were expressed as the area under the curve (AUC) for the 15- to 30 min period (second phase). Data were analyzed using a one-way analysis of variance (ANOVA). In the SNL studies, the force elicited by the von Frey filaments was expressed in grams (g) and analyzed using a repeated-measures ANOVA with treatment as the within-subjects factor. In the Vogel conflict test, total licks were analyzed using a one-way ANOVA with treatment as the between-subjects factor. In the CLS studies, drug effects on both punished and unpunished licking were analyzed using a repeated-measures ANOVA with drug treatment as the within-subjects factor. In the side-effect studies, total distance traveled [locomotor activity (LMA)], time on the rotarod (at both rpm rates), beam walking performance (distance traversed), and body temperature (2 h post-injection) were analyzed using one-way ANOVAs with treatment as the between-subjects factor. Response rate in the FR studies was analyzed using a repeated-measures ANOVA with treatment as a within-subjects factor. In all studies, the significance level for effects of drug was $p<0.05$, and data were analyzed post hoc using Dunnett's test.

Results

Mouse formalin test

MPEP (3–30 mg/kg) and MTEP (1–30 mg/kg) reduced the formalin effect, with MPEP having significant effects at 10 and 30 mg/kg [$F(3,31)=33.0$, $p<0.01$] and MTEP at 3–30 mg/kg [$F(4,38)=5.9$, $p<0.01$]. The 30 mg/kg doses of MPEP and MTEP were also effective during the first phase of the formalin effect, reducing the nociceptive behavior by 20 and 30%, respectively (vehicle=198±12 s, MPEP=160 ±11 s, MTEP=140±11 s). In contrast, the mGluR1 receptor antagonist, LY456236 (10–100 mg/kg), had no effect on the first phase of the response (vehicle=203±11 s, LY456263=220±12 s). However, LY456236 was effective in reducing the second phase response significantly at 30 and 100 mg/kg (Fig. 1) [$F(3,30)=17.7$, $p<0.01$].

Spinal nerve ligation

MPEP (10–30 mg/kg) dose-dependently increased ligated side withdrawal threshold, compared to the vehicle-treated group [$F(24,252)=1.6$, $p<0.05$] (Fig. 2), with a significant increase at 30 mg/kg, which had a peak effect at 30 min and slowly returned to control values within 90 min. MTEP (1–10 mg/kg) produced a dose-dependent increase in withdrawal threshold [$F(24,252)=1.6$, $p<0.05$] with a significant effect at 10 mg/kg (Fig. 2), with a peak effect at 60 min. LY456236 (1–10 mg/kg) also increased withdrawal threshold with the 10 mg/kg dose producing a significant antiallodynic effect [$F(8,76)=4.9$, $p<0.01$]

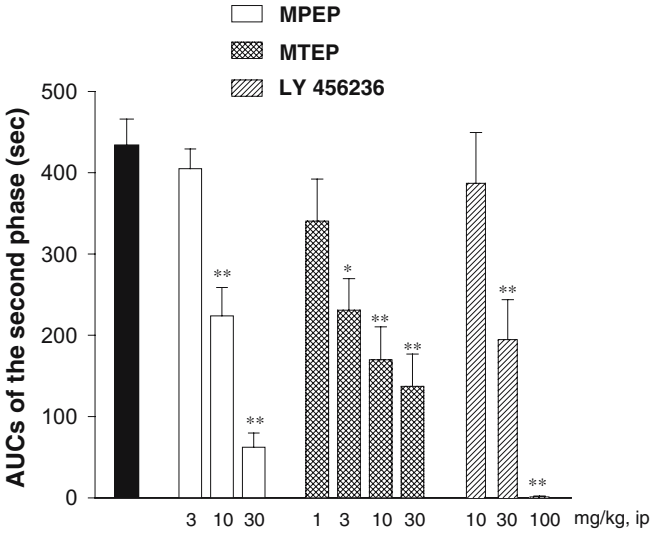

Fig. 1 Antihyperalgesic effect of the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in the formalin test in mice. Formalin (1.5%; 30 μl) was injected subcutaneously into the plantar surface of the right hind paw of the mouse. Time spent licking and flinching the paw was considered a measure of nociceptive intensity. *Bars* represent the area under the curves for the second phase, calculated from 15 to 30 min after formalin injection. Data are mean±SEM of eight to 12 animals per group. *$p<0.05$ and **$p<0.01$ vs vehicle (*solid bar*)

remaining above control values for at least 60 min (Fig. 2). The antiallodynic effects of MPEP and MTEP did not reach the naive threshold (15 g) or at least the contralateral side threshold (Fig. 2). On the contrary, LY456236 completely reversed the allodynia, restoring the threshold to near-naive threshold and above the threshold of the contralateral paw (Fig. 2).

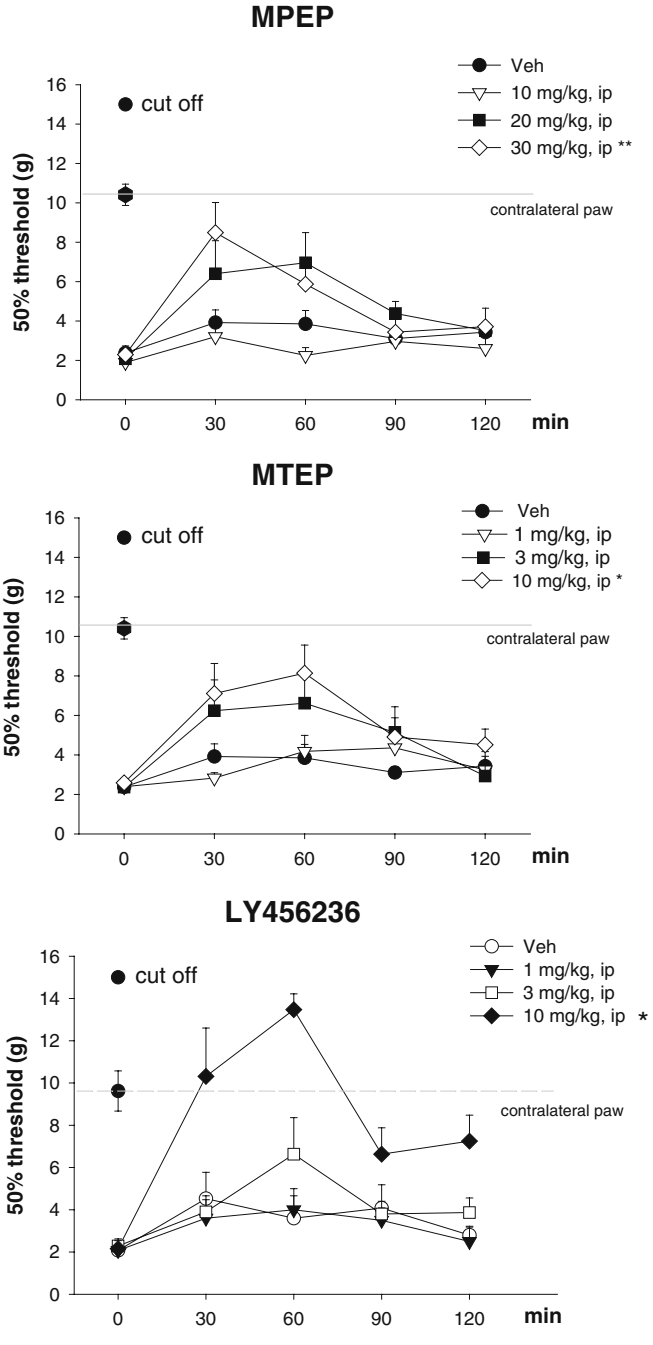

Fig. 2 Antiallodynic effects of mGluR5 antagonists, MPEP and MTEP, and mGluR1 antagonist, LY456236, in L5–L6 spinal nerve ligated rats. Data are mean±SEM of eight to 20 rats and represent the 50% response threshold calculated for each rat. *p<0.05 and **p<0.01 vs vehicle

Vogel conflict

MPEP [F(3,34)=28.7, p<0.01] and MTEP [F(3,67)=8.0, p<0.01] produced anxiolytic-like increases in licking in the Vogel conflict test at 3 and 10 mg/kg (Fig. 3). In both studies, the anxiolytic-like effects of MPEP and MTEP reached the level achieved by the benzodiazepine control, CDP (vehicle=150±27 licks, 1 mg/kg=186±35, 3 mg/kg=269 ±59, 10 mg/kg=627±134). LY456236 also produced an anxiolytic-like increase in licking [F(3,31)=4.6, p<0.01] with a significant effect at 10 mg/kg (Fig. 3). At 30 mg/kg, LY456236 appeared to reduce licking, suggesting that some secondary behavioral effect may be influencing the rats.

Conditioned lick suppression

MPEP and MTEP produced anxiolytic-like increases in licking during the punished phase in the CLS test (Fig. 4). MPEP increased punished licking [F(4,56)=8.0, p<0.01] with a trend at 10 mg/kg and a significant effect at 30 mg/kg; similarly, MTEP increased licking [F(4,44)=18.0, p<0.01] with a trend at 1 mg/kg and significant effects at 3 and 10 mg/kg. MPEP and MTEP had no effect on unpunished licking (Table 1). LY456236 increased punished licking [F(3,45)=6.1, p<0.01] with a significant effect at 30 mg/kg; however, this dose also reduced unpunished licking (Table 1) suggesting that LY456236 may be affecting responding. In all studies, the positive control, CDP (6 mg/kg), increased punished licking (Fig. 4). CDP reduced unpunished licking in all three studies (Table 1), possibly due to its sedative effects. Finally, on an important note, while MPEP and MTEP were able to achieve efficacy of the magnitude of CDP, LY456236 only increased punished licking to ~50% of the CDP response.

Body temperature

MPEP and MTEP significantly reduced body temperature (Table 2), and these reductions were evident 30 min after treatment and persisted for at least 3 h (the maximum length of our study). For brevity, only the body temperature 2 h after injection was analyzed statistically as the maximum effect was observed at this time point. At this time point, MPEP [F(4,54)=18.0, p<0.01] and MTEP [F(5,59)=56.0, p<0.01] significantly reduced body temperature at doses of 10–100 mg/kg (Table 2). In the same design of study, LY456236 had no effect on body temperature (Table 2) up to a dose of 30 mg/kg, across the 3 h test.

Locomotor activity

MPEP [F(4,45)=4.0, p<0.01] and MTEP [F(5,66)=5.7, p<0.01] reduced LMA in terms of the total distance traveled with significant reductions at 100 and 10–100 mg/kg, respectively (Table 2). LY456236 had no effect on LMA up to a dose of 30 mg/kg (Table 2).

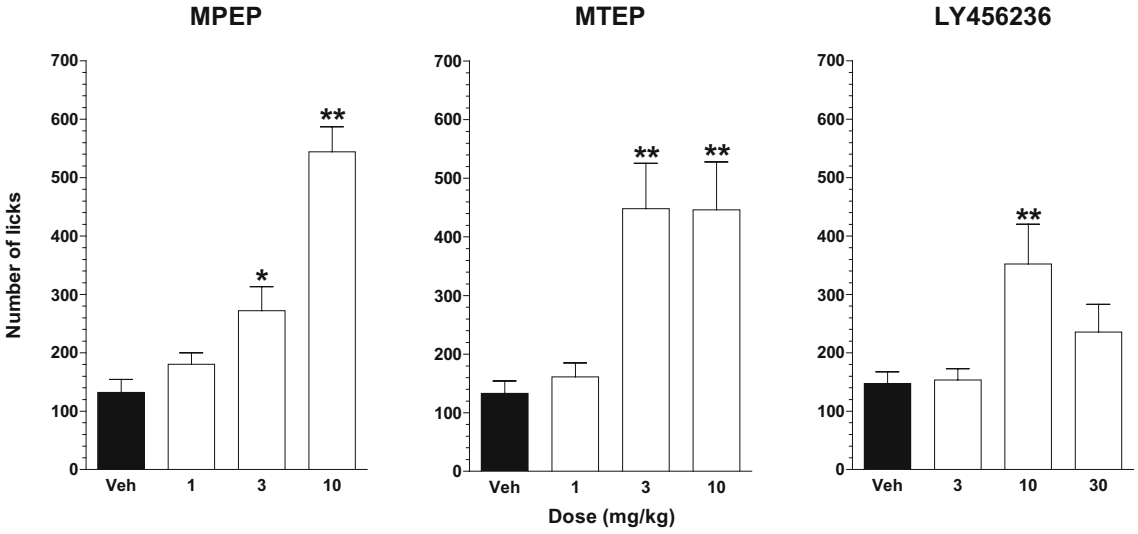

Fig. 3 Anxiolytic-like effects of the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in the rat Vogel conflict test. Data are mean±SEM of 8 to 19 rats and represent the mean number of licks for each rat. *p<0.05 and **p<0.01 vs vehicle

Rotarod

MPEP significantly reduced rotarod performance at both the 8 rpm [F(4,50)=6.6, p<0.01] and 16 rpm [F(4,50)=4.7, p<0.01] speeds. The 30 mg/kg dose reduced performance at 16 rpm, and the 100 mg/kg dose reduced performance at both 8 and 16 rpm (Table 2). MTEP significantly reduced rotarod performance at doses of 30 and 100 mg/kg at both the 8 rpm [F(5,54)=4.0, p<0.01] and 16 rpm [F(5,54)=8.5, p<0.01] speeds (Table 2). LY456236 had no effect on rotarod performance up to a dose of 30 mg/kg (Table 2).

Beam walking

MPEP had no effect on the distance traversed, but MTEP significantly reduced this measure [F(5,54)=3.2, p=0.01] at a dose of 100 mg/kg (Table 2). LY456236 had no effect up to a dose of 30 mg/kg (Table 2).

Fixed-ratio responding

MPEP [F(3,33)=31.0, p<0.01] and MTEP [F(3,44)=9.9, p<0.01] reduced responding in the FR assay with significant reductions at doses of 10–30 and 1–10 mg/kg,

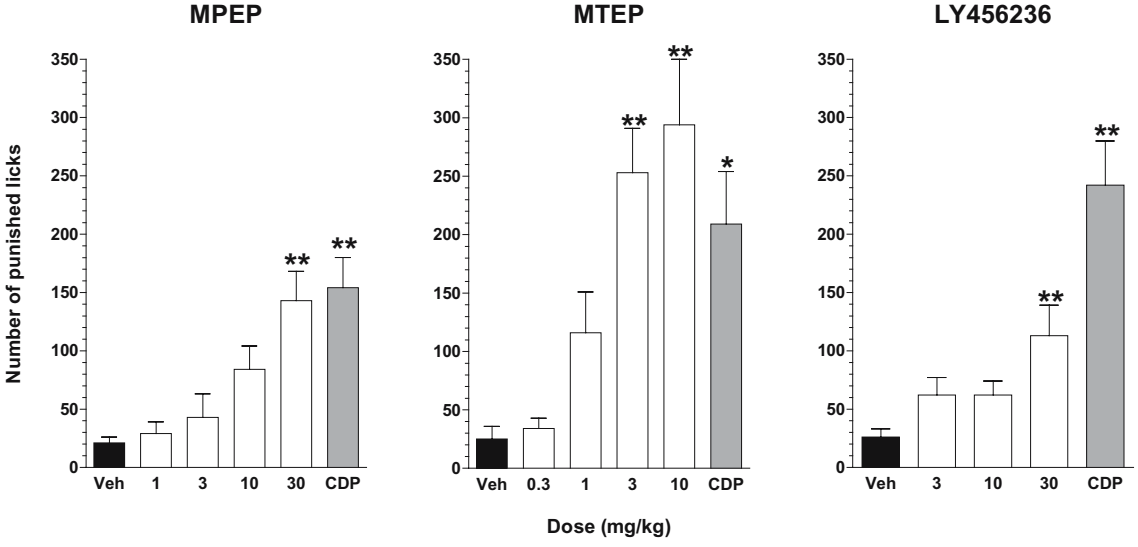

Fig 4 Anxiolytic-like effects of the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in the rat conditioned lick suppression (*CLS*) test. Data are mean±SEM of 12–16 rats and represent the mean number of punished licks for each rat. This study was of a within-subjects design such that each rat served as its own control. *p<0.05 and **p<0.01 vs. vehicle. CDP was included as a positive control and tested at a maximal effective dose of 6 mg/kg

Table 1 Effects of the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, on unpunished licking during the rat conditioned lick suppression (CLS) test

Dose (mg/kg)	MPEP	MTEP	LY456236
0	515±141	428±81	357±103
0.3	NT	410±81	NT
1	536±99	505±122	NT
3	433±97	548±91	264±54
10	344±59	536±94	262±58
30	465±130	NT	135±25*
CDP 6	228±41**	321±72	280±60

Chlordiazepoxide (CDP) was included as a positive control in each CLS test and was tested at a maximal effective dose of 6 mg/kg
NT Not tested
* $p<0.01$ vs vehicle (0 mg/kg)
** $p<0.05$ vs vehicle

respectively (Table 2). LY456236 reduced responding [$F(3,27)=8.5$, $p<0.01$] at a dose of 30 mg/kg (Table 2).

Discussion

A major aim of these studies was to test the effects of group I mGluR antagonists in models of pain and models sensitive to anxiolytic drugs. Specifically, the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, were administered systemically and tested in the acute pain formalin model in mice and the allodynic spinal nerve ligation model in rats. Furthermore, all three drugs were tested in two models sensitive to the effects of clinically used anxiolytic drugs: Vogel conflict and conditioned lick suppression.

We first demonstrated that mGluR5 and mGluR1 antagonists exhibit robust analgesic effects in the mouse formalin test, a nociceptive model that presents both acute (early phase) and chronic (second phase) nociceptive conditions. MPEP, MTEP, and LY456236 dose-dependently reduced the amount of licking and flinching following intraplantar formalin injection including effects during the second phase of the response. Furthermore, the three antagonists reduced hyperalgesia by a similar magnitude. These results are consistent with previous studies that have implicated both mGluR5 and mGluR1 in mediating the noxious effects of formalin (Bhave et al. 2001; Zhou et al. 2001; Noda et al. 2003). Therefore, our findings support the potential utility of group I mGluR antagonists as analgesics for chronic pain.

Additionally, using the rat SNL model, we demonstrated that MPEP, MTEP, and LY456236 reduced the development of mechanical allodynia. These data support the role of group I mGluRs in mediating aspects of chronic, neuropathic pain (Fundytus et al. 1998, 2001; Hofmann et al. 2001) and are consistent with findings from previous studies (Dogrul et al. 2000; Yashpal et al. 2001; Sotgiu et al.

Table 2 Effects of the mGluR5 antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, on body temperature, locomotor activity (distance traveled), rotarod and beam walking performance, and fixed-ratio (FR) responding in the rat

Drug dose (mg/kg)	Body temperature (°C)	Distance traveled (cm)	Rotarod		Beam walk (cm)	FR responding
			8 rpm	16 rpm		
MPEP						
0	37.5±0.1	2,634±536	117±3	110±8	85±5	1.26±0.06
3	37.1±0.2	1,672±254	120±0	112±5	90±0	1.22±0.07
10	36.7±0.1*	1,371±553	120±0	110±7	83±7	0.77±0.07*
30	36.6±0.1*	1,411±377	111±8	79±11**	90±0	0.62±0.09*
100	35.6±0.3*	496±92*	80±12*	64±16*	85±4	NT
MTEP						
0	37.3±0.1	3,695±586	120±0	120±0	90±0	1.03±0.13
1	37.4±0.1	3,408±559	112±7	110±10	90±0	0.73±0.10**
3	37.0±0.1	2,422±278	114±6	98±12	90±0	0.58±0.08*
10	36.7±0.1**	2,048±582**	110±7	94±13	81±8	0.32±0.05*
30	35.2±0.3*	2,087±225**	89±12**	57±13*	90±0	NT
100	33.1±0.4*	1,393±139*	71±13*	31±11*	70±6*	NT
LY456236						
0	37.3±0.2	1,572±214	118±2	93±13	90±0	1.18±0.12
3	37.5±0.1	1,818±333	120±0	110±7	90±0	1.26±0.14
10	37.4±0.2	1,155±233	120±0	112±7	90±0	1.09±0.12
30	37.5±0.1	1,811±204	109±11	101±10	90±0	0.71±0.12*

MPEP: n=11–12 (body temperature), 10 (LMA), 11 (rotarod), 11 (beam walk), 12 (FR); MTEP: n=10–11 (body temperature), 12 (LMA), 10 (rotarod), 10 (beam walk), 12 (FR); LY456236: n=10 (body temperature), 12 (LMA), 10 (rotarod), 10 (beam walk), 10 (FR)
NT Not tested
* $p<0.01$ vs vehicle (0 mg/kg)
** $p<0.05$ vs vehicle

2003; Urban et al. 2003). Interestingly, only LY456236 was able to completely attenuate the allodynic response back to control levels. While MPEP and MTEP were able to attenuate the effects of SNL, neither compound was able to completely reverse the allodynia. These findings support the idea that mGluR1 and mGluR5 have differential roles in mediating nociceptive responses (Neugebauer et al. 2003; Li and Neugebauer 2004). Therefore, based on our comparative studies, mGluR1 antagonists may be more effective than mGluR5 antagonists in treating neuropathic pain, although further studies with a broader range of compounds are warranted to confirm this.

In the second part of these studies, MPEP, MTEP, and LY456236 were tested in two models sensitive to anxiolytic drugs based on studies that have implicated both mGluR5 and mGluR1 in anxiety disorders and have suggested that mGluR5 (Klodzinska et al. 2000; Spooren et al. 2000; Tatarczynska et al. 2001; Schulz et al. 2001; Brodkin et al. 2002a; Pilc et al. 2002) and mGluR1 antagonists (Klodzinska et al. 2004a) may represent novel anxiolytic-like drugs with a reduced propensity to induce benzodiazepine-like side effects. Our studies confirmed the potent anxiolytic-like effect of MPEP in the Vogel conflict model (see Tatarczynska et al. 2001; Pilc et al. 2002) at doses that are known to bind to the mGluR5 receptor (Anderson et al. 2002). Further, we extended this finding to the structurally related mGluR5 antagonist, MTEP (see also Klodzinska et al. 2004b), which produced a significant anxiolytic-like effect in this model and, additionally, appeared to be slightly more potent than MPEP. Given the similar in vitro binding affinity for MPEP and MTEP at the mGluR5 receptor, the increased in vivo potency of MTEP may be due to an improved pharmacokinetics profile, for example, improved bioavailability and/or brain penetration. Importantly, the anxiolytic-like effects of MPEP and MTEP in the Vogel conflict model were similar in magnitude to the benzodiazepine CDP. The anxiolytic-like effects of MPEP and MTEP were also observed in the CLS model, a conditioning model similar to the Vogel that requires the rat to suppress licking behavior during the presentation of a light cue previously associated with mild foot shock. In the CLS test, MPEP and MTEP produced a robust increase in licking during the punishment phase, and again, the magnitude of effect was similar to that seen with a maximally effective dose of CDP. Importantly, although shock is initially used to condition the rats in the CLS test, no shock is presented during the "punished phase" of the test. Therefore, the anxiolytic-like effects of MPEP and MTEP were not due to any potential analgesic effect. Additionally, we have tested the mu opioid agonist, morphine, in both the Vogel and CLS assays, and it was ineffective at analgesic doses (data not shown). Therefore, in line with previous studies (Schulz et al. 2001; Brodkin et al. 2002a), we have demonstrated that MPEP can produce anxiolytic-like effects in conditioned and unconditioned response models, similar to the benzodiazepines, and we have extended this finding to include the newer mGluR5 antagonist, MTEP.

LY456236 also produced anxiolytic-like effects in the Vogel conflict and CLS models. These data support recent findings of Klodzinska et al. (2004a), who demonstrated that the systemic administration of the mGluR1 antagonist, AIDA, produced anxiolytic-like effects in the elevated plus maze and Vogel conflict assays. However, in our study, the anxiolytic effects of LY456236 were smaller in magnitude, compared to MPEP, MTEP, and CDP. Furthermore, in both models, there was evidence that the 30 mg/kg dose of LY456236 affected behavior; total licking was reduced in the Vogel conflict study, and licking during the unpunished phase of the CLS test was reduced. However, given the lack of effect of LY456236 on LMA, this might suggest that the reduced licking following LY456236 is not due to sedation-like effects and may be related to a reduced motivation to lick. Further studies are needed to explore this.

When compounds affect motor coordination or produce sedation, these effects may confound the interpretation of data from behavioral studies, including data from the pain and stress/fear models used in these studies. Spooren et al. (2000) and Schulz et al. (2001) addressed potential side effects following the systemic administration of MPEP by examining spontaneous LMA and reported no significant effects up to doses of 100 and 30 mg/kg, respectively. Conversely, Brodkin et al. (2002a) found that MPEP significantly decreased the rate of responding in the unpunished component of the Geller–Seifter assay. No data with LY456236 are available in the literature, although the mGluR1 antagonist, AIDA, neither induced sedation nor disturbed motor coordination in rats (Klodzinska et al. 2004a). As secondary effects can influence behavior and will ultimately determine the potential safety margin for mGluR antagonists, MPEP, MTEP, and LY456236 were tested in five assays measuring motor coordination, response rate, and body temperature in order to determine the potential issues with mGluR5 or mGluR1 antagonists and to establish safety margins. MPEP and MTEP produced effects in the assays at dose ranges of 10–100 and 1–100 mg/kg, respectively. LY456236 only reduced FR responding at a dose of 30 mg/kg; there were no effects of LY456236 in the other four assays up to a dose of 30 mg/kg. By comparing efficacious doses to doses that affected secondary behaviors, MPEP and MTEP exhibited little, if any, window between efficacious doses in the SNL and Vogel conflict models and doses producing potential side effects. LY456236, on the other hand, exhibited approximately a ten-fold window between the antiallodynic and anxiolytic-like dose and a dose that would potentially produce side effects. However, it is important to note that our studies have examined only a limited number of potential side effects. Other potential issues, including effects on learning and memory, effects on prepulse inhibition that have been reported with both mGluR1 (Brody et al. 2003) and mGluR5 (Kinney et al. 2003; Brody et al. 2004) knockout mice, and interactions between mGluR5 antagonists and psychomimetics (see Kinney et al. 2003), need to be addressed further. Furthermore, it will be important to establish whether mGluR5 and mGluR1 antagonists ex-

hibit some of the issues associated with the benzodiazepines, particularly tolerance following chronic dosing and withdrawal syndrome following drug cessation.

Importantly, the full pharmacokinetic profile of LY45 6236 is unknown. The studies of Anderson et al. (2002, 2003) demonstrate a dose proportional occupancy of mGluR5 receptors within the CNS after intraperitoneal administration of MPEP and MTEP, with doses of 10 and 3 mg/kg of each, respectively, achieving >75% receptor occupancy. However, to the best of our knowledge, equivalent occupancy data for LY456236 binding to mGluR1 following systemic administration are not available. Accordingly, it is difficult at the present time to compare the efficacy and tolerability profile of an mGluR1 antagonist compared with mGluR5 antagonists. The data of Anderson et al. (2002, 2003), together with the present efficacy data, however, might suggest that, at least in the mGluR5 subclass, there is little separation between anxiolytic and motor and/or motivational side effects, at least following an acute dosage. Clearly, a broader range of tool compounds is required to further characterize mGluR5 and mGluR1.

In conclusion, mGluR5 and mGluR1 antagonists are effective in rodent models of anxiolysis and nociception. While mGluR5 antagonists elicit more robust anxiolytic-like effects compared to the mGluR1 antagonist LY456236, the opposite may be true for the antinociceptive properties of these agents. Future studies with antagonists from structurally diverse chemical series with well-characterized pharmacokinetic profiles, as well as studies in alternative species, will ultimately test the generality of these observations.

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