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Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats

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Abstract

The involvement of Group II metabotropic receptors in acute and persistent pain states was evaluated in several in vivo models of pain with selective and potent Group II metabotropic glutamate (mGlu) 2,3 agonists. LY354740, LY379268 and LY389795 attenuated late-phase paw-licking pain behavior in a dose-dependent manner in the formalin model of persistent pain. Effects occurred in the absence of overt neuromuscular deficits as measured by performance in the rotorod test for ataxia. The effects of LY354740 and LY379268 were also stereoselective. The order of potency of the agonists was LY389795 > LY379268>LY354740. The attenuation of licking behavior by LY379268 (3 mg/kg) in the formalin model was reversed by a potent and selective mGlu2,3 receptor antagonist, LY341495 (1 mg/kg). In the L_5/L_6 spinal nerve ligation model of neuropathic pain in rats, LY379268 significantly reversed mechanical allodynia behavior in a dose-related manner. In contrast, LY379268 had no significant effects on the tail flick test or paw withdrawal test of acute thermal nociceptive function. These results support the involvement of Group II mGlu2,3 receptors in persistent pain mechanisms and suggest the potential utility of selective Group II mGlu agonists for the treatment of persistent pain.

Keywords: mGlu2,3 receptors; Neuropathic pain; Persistent pain; LY354740; LY379268; LY389795

1. Introduction

Glutamate receptors play a significant role in the processing of nociceptive information in the central nervous system (CNS) and in the excitability of spinal pain-transmitting neurons. There is evidence indicating that persistent stimulation of glutamatergic neurons in pain pathways by intense nociceptive stimulation results in central sensitization and the development of neuroplasticity in spinal neurons in animals as well as in humans (Woolf and Thompson, 1991; Coderre et al., 1993a; Urban et al., 1994). These mechanisms are postulated to contribute to persistent clinical pain.

Several studies have shown that the modulation of glutamate receptor function via glutamate antagonists can block the facilitation of nociceptive processing produced by either prolonged nociceptive stimulation or direct C-fiber activation (Davies and Lodge, 1987; Dickenson and Sullivan, 1987; Haley et al., 1990; Woolf and Thompson, 1991; Coderre et al., 1993a; Urban et al., 1994). Modulation of glutamate via blockade of glutamate receptors has thus been proposed as a novel therapeutic approach to treat persistent pain (Urban et al., 1994; Simmons et al., 1998a; Fundytus, 2001).

More recently, the role of metabotropic glutamate (mGlu) receptors in the modulation of spinal excitability has evoked much interest. Studies of the effects of mGlu receptor agonists on responses of spinal cord neurons (Neugebauer et al., 1994; Young et al., 1995; Stanfa and Dickenson, 1998) have suggested that mGlu receptors in the spinal cord might be involved in the induction of long-term enhancement of the response to a painful stimulus. A variety of mGlu receptor subtypes have been shown to be present in dorsal root ganglia (DRG) (Ohishi et al., 1995; Valerio et al., 1997) and dorsal horn of the spinal cord of rats. The development of selective metabotropic receptor agonists and antagonists in recent years (Knopfel et al., 1995; Monn et al., 1996, 1997; Schoepp et al.,1997, 1999; Conn and Pin, 1997; Ornstein et al., 1998a,b; Johnson et al., 1999) has provided important tools to further investigate mGlu receptor modulation of glutamate function in pain processing.

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While a number of recent studies have focused on Group I mGlu receptors in pain (review Fundytus, 2001), less is known about the specific role of Group II mGlu receptors in pain processing. Group II mGlu receptors (mGlu2 and mGlu3) are negatively coupled through G_i to adenylyl cyclase (Hayashi et al., 1992; Schoepp, 1994; Conn and Pin, 1997) and have been implicated in modulating the release of glutamate in central pathways (Monn et al., 1997; Conn and Pin, 1997; Schoepp et al., 1999; Cartmell et al., 2000). Carlton et al. (2001) have shown mGlu2,3-like immunoreactivity in lamina IIi with lighter staining in lamina III and IV in lumbar dorsal horn in the rat and also in L_4 and L_5 DRG cells. In an electrophysiological study, Neugebauer et al. (2000) have shown that spinally administered Group II mGlu agonists attenuated responses of primate STT neurons to mechanical stimuli after application of capsaicin. Studies by Boxall et al. (1998) have suggested that upregulation of mGlu3 receptor mRNA occurs in the spinal cord of the rat following UV irradiation-induced peripheral inflammation while Neto et al. (2000) have shown increased expression of mGlu3 receptors in the reticular thalamic nucleus of monoarthritic rats and administration of a Group II mGluR antagonist, EGLU, into this region attenuated nociceptive responses in these rats (Neto and Castro-Lopes, 2000).

In order to further clarify the role of Group II mGluRs in nociceptive stimulus processing in rats, three potent, highly selective Group II mGlu receptor agonists with varying binding affinities (Table 1) were evaluated in the formalin model of persistent pain (Coderre et al., 1990; Simmons et al., 1998a): LY354740 ([(+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate], a conformationally constrained analog of glutamate (Monn et al., 1997), and two heterobicyclic amino acids, LY379268 [(−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate] and LY389795 [(−)-2-thio-4-aminobicyclo [3.1.0] hexane-4,6-dicarboxylate] (Monn et al., 1999). The effects of all three agonists were also compared in the rotorod test of ataxia. LY379268 was additionally evaluated in the L_5/L_6 nerve ligation model of neuropathic pain (Kim and Chung, 1992) and in the tail flick reflex and paw withdrawal latency tests of acute nociceptive pain (Hargreaves et al., 1988; Bjorkman, 1995; Shannon et al., 1997; Sluka et al., 1999; Kalra et al., 2001).

2. Materials and methods

2.1. Animals

Rats were maintained at constant temperature and light (12 h light/12 h dark) for 4–7 days prior to the studies. All testings were conducted in the light cycle and the testing room temperature was maintained at 21–23 °C. Animals had free access to food and water at all times prior to the day of the experiment. All experiment protocols were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee.

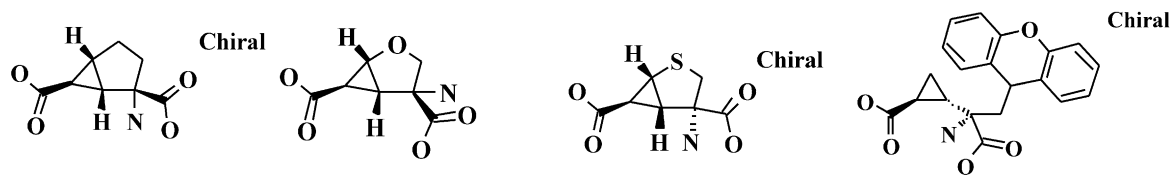
2.2. Drugs and injections

LY354740, LY379268, LY389795, LY366563, LY379267 and LY341495 were synthesized at Eli Lilly and Company (Indianapolis, IN). Drugs or vehicle was administered by intraperitoneal route in a volume of 1 ml/kg

Table 1
Published in vitro binding affinities of Group II mGlu receptor agonists and antagonist

Compound	Rat brain (IC_{50}) [nM]	(h)mGlu2 (K_i) [nM]	(h)mGlu3 (K_i) [nM]
LY354740	180 ± 52[a]	114 ± 19[b]	133 ± 10[b]
LY366563	>100,000[a]	40,300 ± 9600[b]	45,200 ± 12,300[b]
LY379268	15.0 ± 4.0[c]	14.1 ± 1.4[c]	5.8 ± 0.6[c]
LY379267	5040 ± 260[c]	2000 ± 34[c]	1040 ± 89[c]
LY389795	8.4 ± 0.8[c]	40.6 ± 3.7[c]	4.7 ± 1.2[c]
LY341495 (Group II antagonist)		2.3 ± 0.5[b]	1.3 ± 0.2[b]

(h) = recombinant human receptors in RGT cells.
Compound structures:



LY354740 LY379268 LY389795 LY341495

[a] Monn et al. (1997): ACPD-sensitive [^{3}H]glutamate binding.
[b] Johnson et al. (1999): [^{3}H]-LY341495 binding.
[c] Monn et al. (1999): [^{3}H]-LY341495 binding.

with a pretreatment time of 30 min. Vehicle for test compounds was double distilled water.

2.3. Formalin test

The formalin test was performed in custom-made Plexiglas boxes 25 × 25 × 20 cm (length × width × height) height) in size according to Simmons et al. (1998a) based on Shibata et al. (1989) and Wheeler-Aceto et al. (1990). Early (0–5 min) and late (15–40 min) phases of paw-licking behavior were quantitated visually by a blinded observer using an automated behavioral timer, as previously described (Simmons et al., 1998a).

A mirror placed at the back of the box allowed the unhindered observation of the formalin-injected paw. Male Sprague–Dawley Charles River rats (Portage, MI) weighing 200–230 g were acclimatized individually in the cubicles at least 30 min prior to the experiment. Formalin (50 μl of a 5% solution in saline) was injected subcutaneously into the dorsal lateral surface of the right hind paw with a 27-gauge needle. Observation started immediately after the formalin injection. Formalin-induced pain was quantified by recording in 5-min intervals the number of seconds each licking event lasted. These recordings were made for 50 min after the formalin injection, using an automated behavioral timer connected to an IBM PC. Scoring in the formalin test was performed according to Coderre et al. (1993b), Abbott et al. (1995) and Simmons et al. (1998a). The sum of time spent licking (in seconds) from Time 0 to 5 min was considered the early phase, while the late phase was taken as the sum of seconds spent licking from 15 to 40 min.

2.4. L_5/L_6 nerve ligation (Chung model)

Male Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 150–200 g at the time of surgery, were used for these experiments. Surgery was performed as previously described (Kim and Chung, 1992). Briefly, neuropathic injury was produced by tightly ligating the left L_5 and L_6 spinal nerves under gas anesthesia with a mixture of isoflurane (3% for induction and 2% for maintenance) and O_2. Following surgery, development of neuropathic pain was evaluated daily by measuring mechanical sensitivity of the injured paw to von Frey filaments with incremental bending forces (0.5–15 × *g*) as described by Chaplan et al. (1994). Animals were considered to be neuropathic when they exhibited mechanical allodynia, i.e., paw flinch behavior response to the application of a bending force of less than 2 × *g* for 2 days. Test drug or vehicle was administered intraperitoneally and mechanical threshold for paw flinching was measured at 0.5, 1, 2, 3, 4 and 6 h after dosing. Measurement of the mechanical threshold for paw flinching was also done prior to surgery (preoperative control). Data are expressed as the threshold force required to elicit a response (*g*) and are mean ± S.E.M. (standard error of the mean).

2.5. Tail flick reflex

Adult male Sprague–Dawley rats weighing 200–230 g (Harlan) were used for these experiments. The tail flick measurement was made using the Ugo Basile Tail Flick Unit (Ugo Basile, Comerio (VA) 21025, Italy) based on a modification of a method described originally by D'Amour and Smith (1941). The tail flick unit consisted of an infrared heat source (IR source, 50-W bulb) of adjustable intensity that was set at 40 U (determined to elicit tail flick

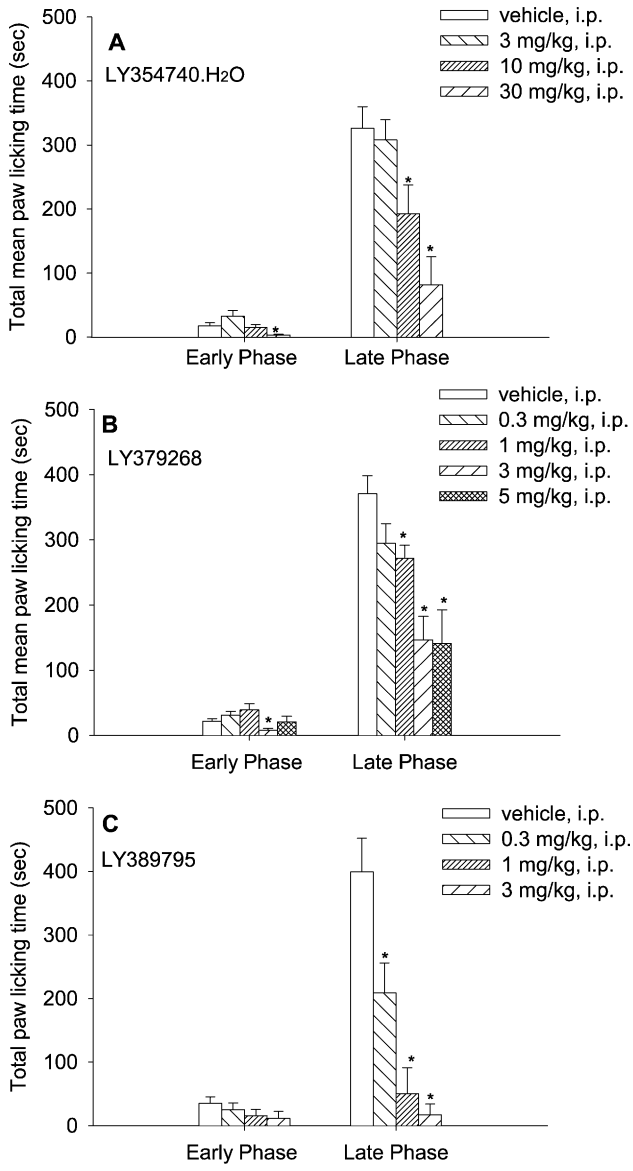


Fig. 1. Effects of intraperitoneally administered mGlu2,3 agonists, expressed as total time spent licking, on formalin-induced early- and late-phase paw-licking behavior. (A) LY354740·H_2O; $n = 8–11$. (B) LY379268; $n = 6–13$. (C) LY389795; $n = 17–18$. Drugs administered 30 min prior to formalin injection. Data are expressed as mean ± S.E.M. and are a result of combining two to three separate experiments for each compound. * $P < .05$ compared to vehicle control group.

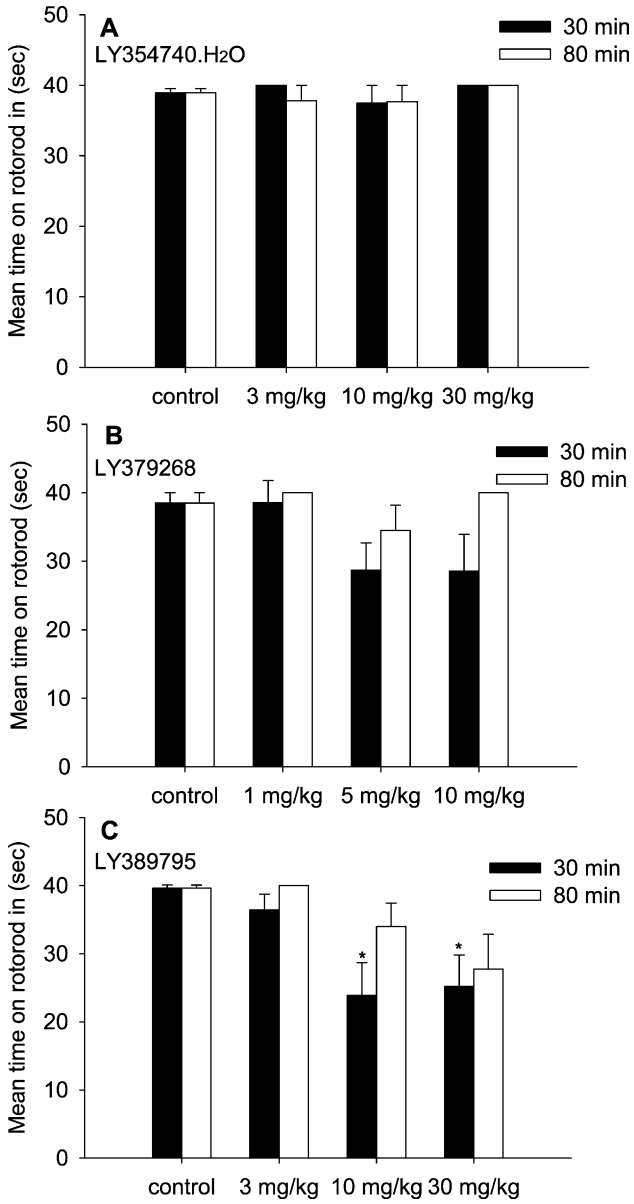


Fig. 2. Effects of mGlu2,3 agonists on rotorod performance, expressed as time spent on rotorod at 30 and 80 min following intraperitoneal administration. (A) LY354740·H_2O. (B) LY379268. (C) LY389795. $n=8$. Mean ± S.E.M. * $P<.05$ compared to control group.

latency of 2–4 s as baseline in naive animals). The IR source was focused to the base of the tail. The latency time (in seconds) required by the rat to reach the thermal threshold for pain and flick its tail was recorded. Each rat was given one test to determine baseline latency to tail flick with a cutoff of 10 s set to avoid tissue damage. Animals were then given drug or vehicle and tested at varying time points. Data were calculated as %MPE (maximum possible effect) and expressed as mean ± S.E.M. %MPE was calculated using the following formula: %MPE=[(Test latency − Baseline latency)/(Cut-off latency (10 s) − Baseline latency)] × 100.

2.6. Paw withdrawal latency

This test measures the nociceptive heat threshold of the rat paw (Hargreaves et al., 1988) in the absence of tissue injury or inflammation. In this test, adult male Sprague–Dawley rats (200–220 g; Harlan) were placed in clear Plexiglas chambers on an elevated glass table and allowed to acclimatize for approximately 5–10 min. The time taken by the rat to withdraw the hind paw in response to a radiant heat source (50-W bulb, Plantar Test Apparatus; Ugo Basile) was measured in seconds to the nearest 0.1 s. A cut-off time of 30 s was maintained to avoid damaging dermal tissue. Both hind paws were measured independently and latency time was averaged. Bilateral measurements of paw withdrawal latency were made prior to drug or vehicle treatment and again 30 min following the intraperitoneal administration of either LY379268 or vehicle. The data were then expressed as a percent of the maximum possible effect (with a maximum of 100%, i.e., thermal threshold of 30 s), using the formula: %MPE=[(Test latency − Baseline latency)/(Cut-off latency (30 s) − Baseline latency)] × 100. The validity (Hargreaves et al., 1988; Kalra et al., 2001) and test–retest reliability of this method have previously been established ($r^2=.7$, $P=.0001$) (Sluka et al., 1999).

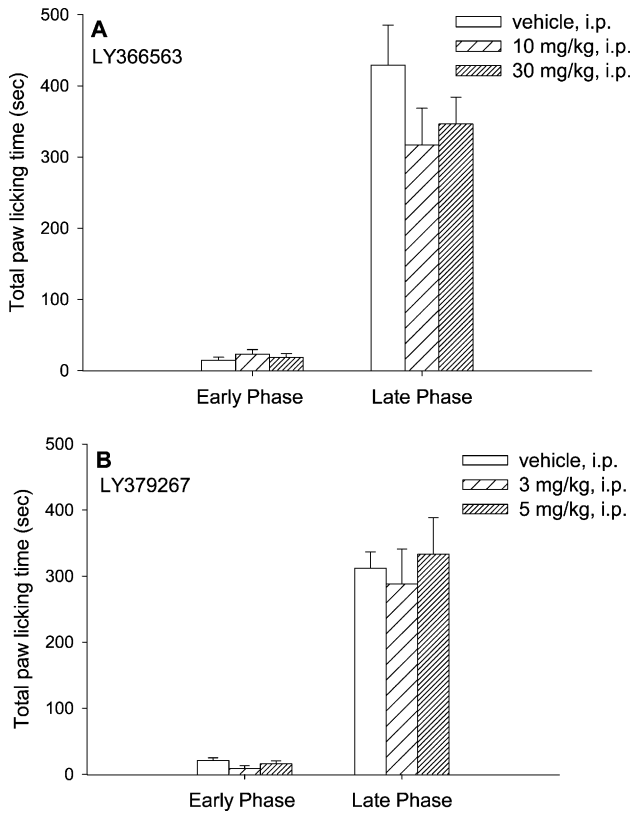


Fig. 3. Effects of (A) LY366563 (opposite enantiomer of LY354740, $n=8$) and (B) LY379267 (opposite enantiomer of LY379268, $n=9–10$) on early- and late-phase formalin-induced paw-licking behavior. Drugs administered 30 min prior to formalin injection. Data expressed as total time spent licking in each phase. Mean ± S.E.M.

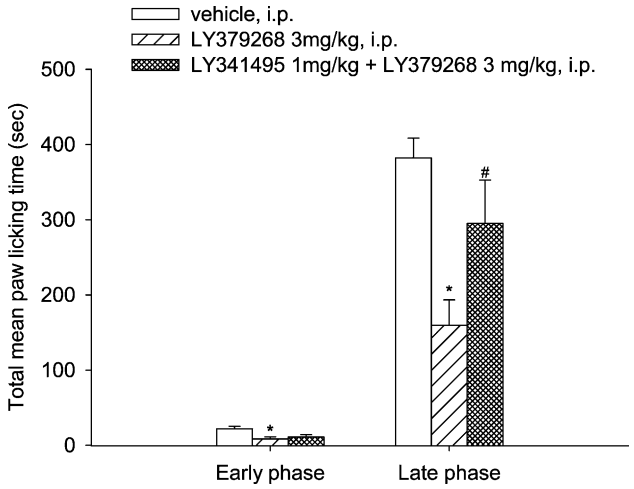


Fig. 4. Reversal of effects of LY379268 with a Group II mGlu receptor antagonist, LY341495, on formalin-induced paw-licking behavior. LY341495 (1 mg/kg) injected concomitantly with mGlu2,3 agonist, LY379268 (3 mg/kg), 30 min prior to formalin. Data expressed as total time spent licking in the early and late phases. $n = 14–21$. Data are expressed as mean ± S.E.M. and are a result of combining three separate experiments. * $P < .05$ compared to vehicle control. # $P < .05$ compared to LY379268, 3 mg/kg group.

2.7. Rotorod test

The ability of various glutamate receptor ligands to induce ataxia was examined using an automated accelerating rotorod (Omnitech Electronics, Columbus, OH) connected to an IBM PC computer (Simmons et al., 1998a). For training and testing purposes, the rotorod was set up to accelerate to 17 rpm in 5 s and maintain that speed for 40 s. Male Sprague–Dawley rats (Charles River) weighing 200–230 g were given three training trials to learn to maintain posture on the rotorod prior to the day of drug testing. The following day, rotorod testing was conducted both at 30 and 80 min following administration of drug. Rats that maintained posture and did not fall off the rotorod were given a maximum score of 40 s.

2.8. Data analysis and statistics for all tests

Data were analyzed by ANOVA and Dunnett's t test (Dunnett, 1964) using JMPv3.2 (SAS Institute, Cary, NC) statistical software. A significance of $P < .05$ was considered to be statistically different from vehicle group. All data are presented as mean ± S.E.M.

3. Results

3.1. Effects of mGlu2,3 agonists in the formalin model of persistent pain

LY354740 was examined for its effects on formalin-induced paw-licking behavior at 3, 10 and 30 mg/kg, 30 min after intraperitoneal administration. LY3547 significantly

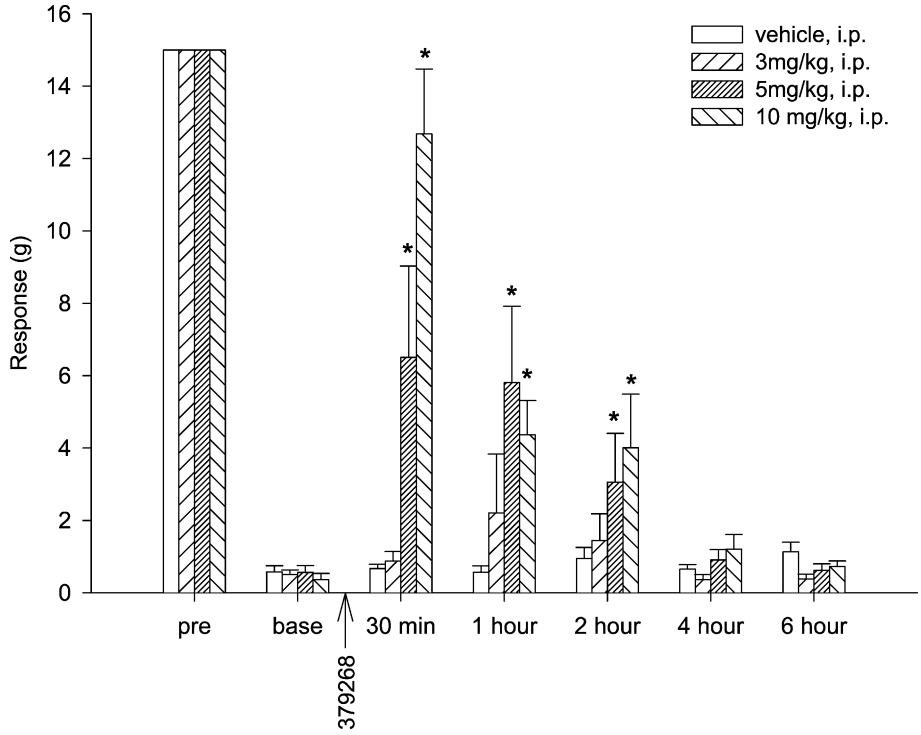


Fig. 5. Effects of LY379268 on mechanical allodynia behavior in rats following L_5/L_6 nerve ligation. Data are expressed as mean ± S.E.M. $n = 7–9$. 'Response (g)' = withdrawal thresholds to bending forces of $0.5–15 \times g$ applied by von Frey filaments. 'Pre' = presurgery response to von Frey filaments. 'Base' = baseline response to von Frey filaments after surgery. * $P < .05$ compared to vehicle group.

decreased the amount of time spent licking in the early (at 30 mg/kg) and late phases (at 10 and 30 mg/kg) (Fig. 1A). LY379268 was tested for its effect on formalin-induced paw-licking behavior at 0.3, 1, 3 and 5 mg/kg ip (30 min). LY379268 caused a reversal of late-phase licking behavior (Fig. 1B) at 1, 3 and 5 mg/kg and had a slight effect on the early-phase licking behavior (at 3 mg/kg) (Fig. 1B). LY389795 also significantly reversed late-phase licking behavior at 0.3, 1 and 3 mg/kg ip with no effect on early-phase licking behavior (Fig. 1C).

The effects of LY354740, LY379268 and LY389795 were dose-related and exhibited an order of potency of LY389795>LY379268>LY354740.

3.2. Effects of mGlu2,3 agonists on performance in the rotorod test of ataxia

No deficits in rotorod performance were observed with LY354740 at doses as high as 30 mg/kg ip (Fig. 2A). No statistically significant deficits in performance were observed with LY379268 up to 10 mg/kg ip. However, at the 30-min time point, there was a decrease in performance time in three of the animals at 5 and 10 mg/kg, which was short-lived and no longer evident at 80 min (Fig. 2B). At the 10 mg/kg dose, some animals also showed a tendency to be easily startled. LY389795 showed no deficits in rotorod performance up to 3 mg/kg ip. However, significant deficits were observed at 10 and 30 mg/kg ip. (Fig. 2C). In the rotorod test as well, the observed order of potency was LY389795>LY379268>LY354740.

Importantly, there were no deficits in rotorod performance at doses of the three agonists where significant reversal of formalin-induced behavior was observed.

3.3. Stereoselectivity of effects of LY354740 and LY379268 on formalin-induced behavior

LY366563, the opposite (−) enantiomer of LY354740, did not have any effect on either phase of formalin-induced paw-licking behavior (Fig. 3A) or on the rotorod test (data not shown) at 10 and 30 mg/kg ip. LY367267, the opposite (+) enantiomer, of LY379268, also did not show any effect on either phase of the formalin test at the doses tested (3 and 5 mg/kg ip) (Fig. 3B). LY379267-treated animals did not show ataxia at those doses as measured by the rotorod test (data not shown). Therefore, the effects of LY354740 and LY379268 were stereoselective, since their opposite enantiomers, which have little or no affinity for mGlu2, 3 receptors (Table 1), did not cause any effects in the formalin or rotorod tests at comparable doses.

3.4. Reversibility of the effect of LY379268 on formalin-induced behavior

The effects of LY379268 at 3 mg/kg ip were reversed when rats were pretreated with LY341495 (1 mg/kg ip; 1–2 min prior to LY379268), a potent and selective mGlu2, 3 receptor antagonist (2*S*-2-amino-2-(1*S*,2*S*-2-carboxycyclopropan-1-yl)-3-(9-xanthyl) propanoic acid) (Kingston et al., 1998; Ornstein et al., 1998a,b) (Fig. 4). These data showed that the effects of LY379268 on formalin-induced behavior were specifically mediated via mGlu2,3 receptors.

3.5. Effects of LY379268 in the L_5/L_6 spinal nerve ligation model of neuropathic pain

In this model of neuropathic pain, L_5/L_6 spinal nerve ligated rats exhibit very significant mechanical allodynia behavior in response to the application of less than $1 \times g$ of mechanical pressure via von Frey filaments. LY379268 (3, 5 and 10 mg/kg ip) significantly increased the threshold for mechanical allodynia behavior in L_5/L_6 spinal nerve ligated rats in a dose-related manner (Fig. 5). Statistically significant effects at both 5 and 10 mg/kg were seen within 30 min after the intraperitoneal administration of the compound. These effects were still evident after 1 h at the 5 mg/kg dose

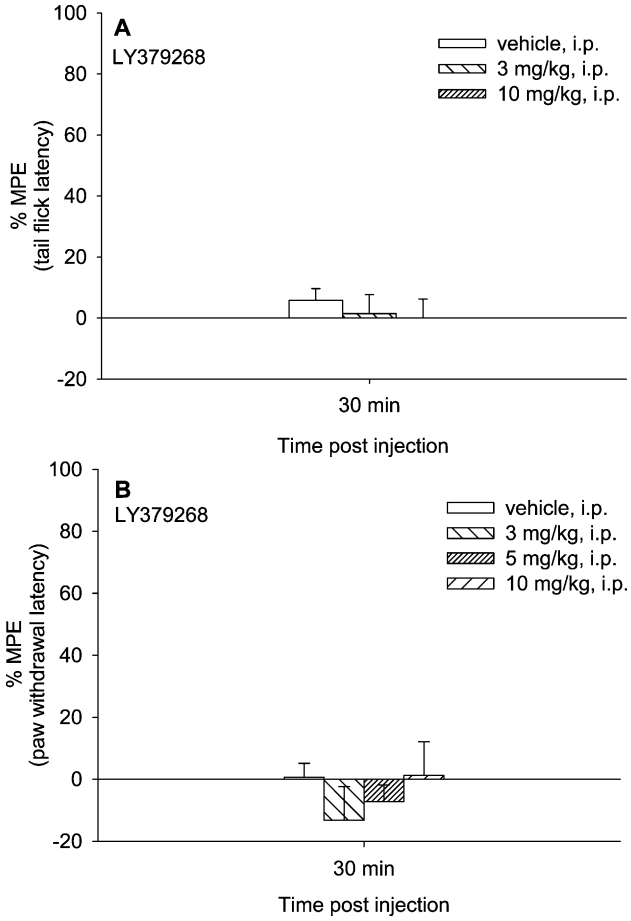


Fig. 6. (A) Effects of LY379268 on tail flick reflex latency, plotted as %MPE (maximum possible effect) and expressed as mean ± S.E.M. $n = 6–7$. (B) Effects of LY379268 on paw withdrawal latency plotted as %MPE (maximum possible effect) and expressed as mean ± S.E.M. $n = 7–8$. %MPE was calculated using the formula: %MPE=[(Test latency − Baseline latency)/(Cut-off latency (30 s) − Baseline latency)] × 100.

and 2 h at the 10 mg/kg dose (Fig. 5), but were no longer evident by 4 h.

3.6. Effects of LY379268 in the tail flick reflex test of acute nociceptive pain

The tail flick test is used to measure loss of pain sensation in the tail. The effects of LY379268 on tail flick latency were evaluated in rats at 3 and 10 mg/kg, 30 min after intraperitoneal administration. No significant effects were noted at either dose tested as compared to vehicle-treated rats (Fig. 6A), indicating a lack of effects of LY379268 on acute nociceptive thermal pain thresholds in this model.

3.7. Effects of LY379268 in the paw withdrawal latency test of acute nociceptive pain

The paw withdrawal latency test is used to measure acute nociceptive pain threshold in the paw in the absence of tissue injury or inflammation. The effects of LY379268 on paw withdrawal latency were evaluated in rats at 3, 5 and 10 mg/kg, 30 min after intraperitoneal administration. No statistically significant effects were observed at any of the doses tested as compared to vehicle-treated rats (Fig. 6B). These data indicate a lack of effects of LY379268 on acute nociceptive thresholds in the normal paw.

4. Discussion

The present study investigated the role of Group II mGlu receptors in several models of persistent and acute pain, including the formalin model of persistent pain, the L_5/L_6 spinal nerve ligation model of neuropathic pain and the tail flick reflex and paw withdrawal models of acute nociceptive pain in rats. The mGlu2,3 agonists had no effect on acute thermal nociception but reduced paw-licking behavior in the formalin model and reduced mechanical allodynia in the neuropathic pain model.

The formalin-induced paw-licking behavior induced by the subcutaneous injection of formalin into the rat hind paw is considered a model of persistent pain and CNS plasticity (Coderre and Melzack, 1992; Coderre et al., 1990). The behavioral response to formalin is biphasic, with an early phase of intense nociceptive behavior (5 min) that is short-lived, followed by an extended tonic response or late phase of exaggerated nociceptive activity lasting up to an hour after the formalin injection (Wheeler-Aceto et al., 1990). The early phase of formalin-induced paw-licking behavior is suggested to measure early sensitization while the late phase seems to be a measure of persistent pain behavior due to central sensitization. Several investigators have suggested the involvement of glutamate receptors in formalin-induced late-phase paw-licking behavior (Coderre and Melzack, 1992; Coderre and Yashpal, 1994; Simmons et al., 1998a). When administered intraperitoneally, all three mGlu2,3 agonists tested, LY354740, LY379268 and LY389795, significantly blocked the late phase of the formalin-induced behavior at doses that did not cause deficits in rotorod performance. These effects were dose-dependent. Moreover, the effects of LY354740 and LY379268 were stereoselective. In addition, the effects of LY379268 were reversed by a selective mGlu2,3 antagonist, LY341495. The order of potency in the formalin model was found to be LY389795>LY379268>LY354740, which was consistent with in vitro binding affinities of the three agonists to rat mGlu2,3 receptors (Table 1). Overall, these results strongly point to a selective modulation of the persistent nociceptive response in the formalin model by Group II mGlu2,3 receptors.

These results are further supported by the significant effects of LY379268 in reversing mechanical allodynia behavior in the L_5/L_6 spinal nerve ligation model (Kim and Chung, 1992). Peripheral nerve injury induces a neuropathic pain state in humans, which includes spontaneous burning, lancinating pain and allodynia (Bonica, 1990). The unique rat model of neuropathic pain described by Kim and Chung (1992), in which the lumbar L_5/L_6 spinal nerves are tightly ligated, has some symptomatic features similar to those described in man: These rats show signs of spontaneous pain, and long-lasting mechanical and cold allodynia behavior that persists for several weeks after surgery. Moreover, this model provides the most consistent lesion of peripheral nerves because of the tight ligation of segmental spinal nerves compared to other models in which the amount of lesion might be more variable. In this model, LY379268 was administered after mechanical allodynia behavior had been established. The significant dose-related reversal of mechanical allodynia behavior by LY379268 in the L_5/L_6 spinal nerve ligation model further supports the involvement of mGlu2,3 receptors in persistent pain mechanisms.

Of specific interest is the lack of effects of LY379268 in two models of acute thermal nociceptive pain, the tail flick test and the paw withdrawal test, at doses that were significantly active in the formalin and nerve ligation models. The rat tail flick and paw withdrawal tests have long been considered models of acute somatosensory pain (Hargreaves et al., 1988; Bjorkman, 1995; Shannon et al., 1997; Sluka et al., 1999; Kalra et al., 2001). These data suggest a difference in the involvement of mGlu2,3 receptors in the processing of acute nociceptive versus persistent nociceptive responses and could have significant therapeutic implications for the treatment of persistent pain states.

It should be noted, however, that a preliminary abstract (Simmons et al., 1998b) had reported the activity of mGlu2,3 agonists in hot plate-induced paw-lick responses and activity in the hot plate model has also been suggested to imply potential utility in acute pain states (Shannon et al., 1997). The reason for the discrepancy between the present results using selective activation of acute thermal nocicep-

tive responses, both in the tail as well as in the paw, and the previously reported studies is not yet clear. It is possible that the hot plate test measures thermal responses in a non-specific manner or that the hot plate paw-licking response is mediated by central circuits differently from that of the tail flick and paw withdrawal thermal responses.

The effects of LY389795 and LY379268 at higher doses in the rotorod test are consistent with previous observations by Cartmell et al. (2000). The latter authors showed that after acute oral administration, LY379268 caused short-lived rotorod deficits that were not evident after sub-chronic dosing.

Results from the present behavioral study in the rat are consistent with a recent report in which Neugebauer et al. (2000) have shown that Group II mGlu agonists, including LY379268, applied spinally via microdialysis, attenuated electrophysiological responses of primate STT neurons to mechanical stimuli such as brush and pinch after application of capsaicin, but not to mechanical stimuli in naïve controls. In addition, Neugebauer and Li (2001) also recently reported that application of LY354740 by microdialysis to the central amygdala could block electrophysiological responses in a model of arthritis in rat. Thus, our results, in conjunction with the latter reports, point to the involvement of mGlu2,3 receptors in persistent pain states induced by a variety of stimuli.

In contrast to the above studies, Boxall et al. (1998) have suggested that upregulation of mGlu3 receptor mRNA occurs in the spinal cord of the rat following UV irradiation-induced peripheral inflammation, while Neto et al. (2000) have shown increased expression of mGlu3 receptors in the reticular thalamic nucleus of monoarthritic rats. Stereotaxic administration of a Group II mGluR antagonist, EGLU, into the thalamic reticular nucleus resulted in attenuation of nociceptive responses in the CFA model of monoarthritis (Neto and Castro-Lopes, 2000). It is difficult to separate effects mediated via mGlu2 or mGlu3 sub-types with currently available tools, which are mixed mGlu2, 3 agonists. Discovery of more subtype-selective metabotropic receptor agonists will help clarify the selective involvement of mGlu2 versus mGlu3 subtypes of metabotropic receptors in various pain transmission processes.

In summary, the present studies support the contention that Group II mGlu receptors play a significant role in the processing of sensory information in pain pathways. Furthermore, the activity of the three agonists observed in the persistent pain behavioral tests suggests that Group II mGlu receptor agonists may be therapeutically useful for the treatment of persistent pain states.

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