



## Peripheral group II metabotropic glutamate receptors mediate endogenous anti-allodynia in inflammation

Dongni Yang<sup>a</sup>, Robert W. Gereau IV<sup>a,b,\*</sup>

<sup>a</sup>Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA

<sup>b</sup>Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Received 28 May 2003; received in revised form 5 August 2003; accepted 26 August 2003

### Abstract

We previously demonstrated that activation of peripheral group II mGluRs inhibits PGE2-induced thermal hyperalgesia. In the present study we examined the role of peripheral group II mGluRs in inflammation-induced mechanical allodynia in CD1 mice. Subcutaneous injection of group II mGluR agonists or antagonists into the plantar surface of the mouse hind paw did not alter mechanical thresholds, suggesting that peripheral group II mGluRs did not modulate basal mechanical sensation. We then used either PGE2 or carrageenan to induce mechanical allodynia and investigated the effects of activating or inhibiting peripheral group II mGluRs. PGE2-injected mice showed an  $87 \pm 1\%$  decrease of mechanical thresholds 75 min after the injection, whereas mice injected with group II mGluR agonists had no increase in sensitivity compared to vehicle-injected mice. In the carrageenan-induced inflammation model, 3 h after carrageenan injection the mechanical thresholds of mice injected with group II mGluR agonist APDC fully recovered to baseline levels while vehicle-injected mice showed only  $43 \pm 8\%$  recovery. The application of group II mGluR antagonist (LY341495) alone delayed the recovery of PGE2- and carrageenan-induced mechanical allodynia. Three hours after injection of carrageenan, LY341495-injected mice showed little or no recovery with mechanical thresholds  $8 \pm 1\%$  of pre-carrageenan baselines, compared to  $57 \pm 8\%$  of pre-carrageenan baselines in vehicle-injected mice at the same time point. Our results suggest that activation of peripheral group II mGluRs reduces inflammation-induced mechanical allodynia and that peripheral group II mGluRs may mediate endogenous anti-allodynia effects, which speed recovery from inflammation-induced hypersensitivity.

© 2003 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

**Keywords:** Mechanical; Carrageenan; PGE2; Inflammation; Nociception; mGluR

### 1. Introduction

Inflammation frequently leads to enhanced pain sensitivity, which includes both hyperalgesia (a decrease in threshold or increase in responses to supra-threshold noxious stimuli), and allodynia (the development of nociceptive responses to previously non-noxious stimuli). During inflammation, a number of substances, collectively termed the 'inflammatory soup', are released in the periphery and contribute to the development of hyperalgesia and allodynia (Levine and Reichling, 1999). One important component of this inflammatory soup is the excitatory neurotransmitter, glutamate (deGroot et al., 2000; Karim et al., 2001; Nordlind et al., 1993; Omote et al., 1998). Glutamate present at the inflammation site can modulate

nociception by directly activating its ionotropic receptors (Chen et al., 1999; Du et al., 2003; Wang et al., 2000) and metabotropic receptors on primary afferent nociceptors (Bhave et al., 2001; Hu et al., 2002; Walker et al., 2001; Yang and Gereau, 2002; Zhou et al., 2001).

The metabotropic glutamate receptors can be divided into three groups based on sequence homology and pharmacological properties (Conn and Pin, 1997). Group I mGluRs (mGluR1/5) are coupled to  $G_{q/11}$ . Groups II (mGluR2/3) and III mGluRs (mGluR4, 6, 7, 8) are coupled to  $G_i$ . At least two groups of mGluRs: groups I and II, are expressed on peripheral sensory axons (Bhave et al., 2001; Carlton et al., 2001; Walker et al., 2001; Zhou et al., 2001). Although activation of peripheral group I mGluRs is pronociceptive (Bhave et al., 2001; Hu et al., 2002; Walker et al., 2001; Zhou et al., 2001), the activation of group II mGluRs appears to be anti-nociceptive. Systemic or

\* Corresponding author. Tel.: +1-713-798-5392; fax: +1-713-798-3946.  
E-mail address: rgereau@bcm.tmc.edu (R.W. Gereau IV).

intrathecal application of group II mGluR agonists produces anti-nociceptive effects (Dolan and Nolan, 2000; Fisher et al., 2002; Sharpe et al., 2002; Simmons et al., 2002). Group II mGluRs are expressed in spinal cord dorsal horn (Boxall et al., 1998; Jia et al., 1999; Ohishi et al., 1993), where their activation can depress synaptic transmission (Gerber et al., 2000) and modulate the response of spinalthalamic tract (STT) cells to noxious stimuli (Neugebauer et al., 2000). In the periphery, the activation of group II mGluRs does not alter paw withdrawal latency to painful heat stimuli. However, co-injection of a group II mGluR agonist blocks prostaglandin E2 (PGE2)-induced thermal hyperalgesia, potentially through inhibition of PGE2-induced sensitization of the heat sensing protein, TRPV1 (vanilloid receptor 1, also known as VR1) (Yang and Gereau, 2002). In this study, we investigated the role of peripheral group II mGluRs (mGlu2/3) in basal mechanical sensation and during inflammation-induced mechanical allodynia in both the PGE2- and carrageenan-induced inflammation models.

## 2. Materials and methods

### 2.1. Behavioral analysis

All studies described here were approved by the institutional animal care and use committee at Baylor College of Medicine, which operates an AAALAC—accredited animal program. These studies are consistent with the policy of the IASP Committee for Research and Ethical Issues. Male CD1 mice (6–8 weeks old) were purchased from the Baylor College of Medicine animal facility. Mechanical sensitivity was measured using von Frey filaments (North Coast Medical, Inc. San Jose, CA). Mice were placed in Plexiglas testing boxes ( $10 \times 10 \times 21 \text{ cm}^3$ ) with a  $1 \times 1 \text{ cm}^2$  wire-mesh grid floor (IITC Life Sciences, Woodland Hill, CA), and habituated for 3 h before experiments. Each von Frey hair was applied to the mouse hind paw until bent at about  $30^\circ$  for about 3 s. The smallest hair that evoked a paw withdrawal response was taken as the mechanical threshold. Similar sites were selected for measuring mechanical thresholds in all tested animals and for each individual animal the thresholds are measured at approximately the same site throughout the experiment. Two to three baselines were measured before drug injection, and the average was calculated as the baseline. All drugs were injected subcutaneously into the plantar surface of the hind paw in a volume of  $10 \mu\text{l}$  using a  $3/10 \text{ cm}^3$  insulin syringe U-100 with  $29_{\text{G}}1/2$  needle (Becton Dickinson, Franklin Lakes, NJ). The needle was inserted near the toes and advanced toward the heel. All drug injections were performed at approximately the same site. In experiments where PGE2 was used, PGE2 (0.01 mg/ml,  $10 \mu\text{l}$ ) or vehicle1 (PBS + 0.1% ethanol) was injected at time 0. At 15 min, (2*R*,4*R*)-4-aminopyrrolidine-2,3-dicarboxylate

(APDC), vehicle2 (PBS + 0.4 mM NaOH + 0.4 mM HCl), APDC + (2*s*)-2-amino-2-[(1*s*,2*s*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495) or LY341495 alone was injected. In experiments where carrageenan was used, carrageenan (2%) was injected at 0 min. APDC, LY341495 or PBS was injected at 55 min.

Mouse paw thickness was measured using a dial caliper with 0.02 mm graduation (Chicago Brand, Fremont, CA). Two points on the top and bottom of the foot were marked so that the thickness was measured at the same point. Three measurements were made for each time point and were averaged. After measuring baseline, carrageenan was injected at time 0. Paw thickness was measured 45 min after significant mechanical allodynia had already developed. Vehicle (PBS + 0.4 mM NaOH + 0.4 mM HCl) or APDC (0.4 mM) was then injected at 55 min and paw thickness was measured at 90, 120 and 180 min.

### 2.2. Drugs

APDC and LY341495 were purchased from Tocris Cookson (Ballwin, MO). Stock solutions of 20 mM APDC and 1 mM LY341495 were made in 1 and 1.2 equiv. NaOH, respectively. A stock solution of 10 mg/ml prostaglandin E2 (PGE2 Sigma) was made in 100% ethanol. All drugs were diluted in phosphate buffered saline solution (PBS, pH 7.4). An equal amount of HCl was added when diluting APDC to adjust pH. Appropriate vehicles were prepared as the diluents for each drug.

### 2.3. Data analysis

Data are expressed as mean  $\pm$  SEM. Treatment effects on PGE2-induced mechanical allodynia were analyzed using one-way ANOVA followed by Tukey's post-hoc multiple comparisons. All time courses of drug effects were analyzed using the two-way ANOVA followed by Bonferroni post-tests when applicable. Error probabilities of  $P < 0.05$  were considered statistically significant. All statistical analyses were done using the Graphpad Prism software.

## 3. Results

### 3.1. Activation of group II mGluRs inhibits PGE2-induced mechanical sensitization

Our previous studies suggested that activation of peripheral group II mGluRs does not modulate basal thermal response threshold but inhibits PGE2-induced thermal hyperalgesia (Yang and Gereau, 2002). To test whether peripheral group II mGluRs have similar effects in mechanical sensation, PBS, APDC (400  $\mu\text{M}$ ,  $10 \mu\text{l}$ ) or

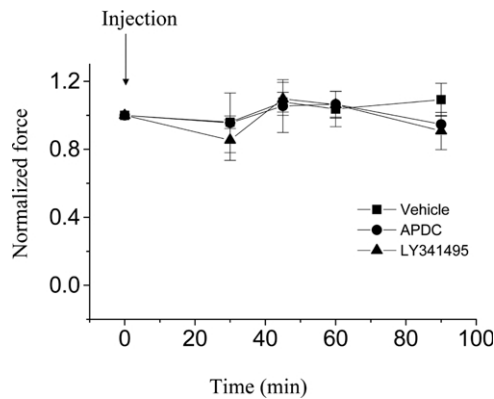


Fig. 1. Group II mGluRs do not modulate mechanical sensation in naïve mice. Graph shows the normalized mechanical thresholds measured over time. Three to five measurements before injection were averaged as baseline, to which all other measurements were normalized. Vehicle (PBS, 10  $\mu$ l), APDC (0.4 mM, 10  $\mu$ l) or LY341495 (0.02 mM, 10  $\mu$ l) was injected subcutaneously into the plantar surface of mouse hind paw at time 0. No significant change was observed in any of the injection groups (two-way ANOVA),  $n = 6-8$ .

LY341495 (20  $\mu$ M, 10  $\mu$ l) was injected subcutaneously into the hind paw of 7–8 weeks old male CD1 mice. Von Frey filaments were used to measure mechanical sensitivity. As shown in Fig. 1, neither APDC nor LY341495 changed mechanical thresholds.

We then used PGE2 to induce mechanical allodynia and investigated the effects of activating peripheral group II mGluRs. At time 0, either vehicle1 or PGE2 was injected, at time 15 min, vehicle2, APDC or APDC + LY341495 was injected. Vehicle1-, vehicle2-injected mice showed allodynia in the first hour after injection. At 75 min, the vehicle effects attenuated so that the mechanical thresholds had returned to baseline levels. We thus choose the 75 min time point to compare the effects of different treatments. As previously reported (Ahlgren and Levine, 1993; Khasar et al., 1993), PGE2 injection induced mechanical allodynia (Fig. 2a, Table 1). Subcutaneous injection of the selective group II mGluR agonist APDC (400  $\mu$ M, 10  $\mu$ l) reduced PGE2-induced mechanical allodynia to the vehicle level (Fig. 2b, Table 1). This APDC effect was blocked by co-injection of LY341495 (20  $\mu$ M, 10  $\mu$ l), a potent group II mGluR antagonist.

Interestingly, we found that at 120 min, in mice injected with APDC and LY341495, mechanical allodynia was significantly exacerbated compared to mice injected with PGE2 and vehicle2 (Fig. 2c). These results suggest an anti-allodynia effect of endogenously activated group II mGluRs. To test this, we injected LY341495 (20  $\mu$ M, 10  $\mu$ l) alone after PGE2 injection. The injection of LY341495 significantly slowed the recovery from PGE2-induced mechanical allodynia (Fig. 3).

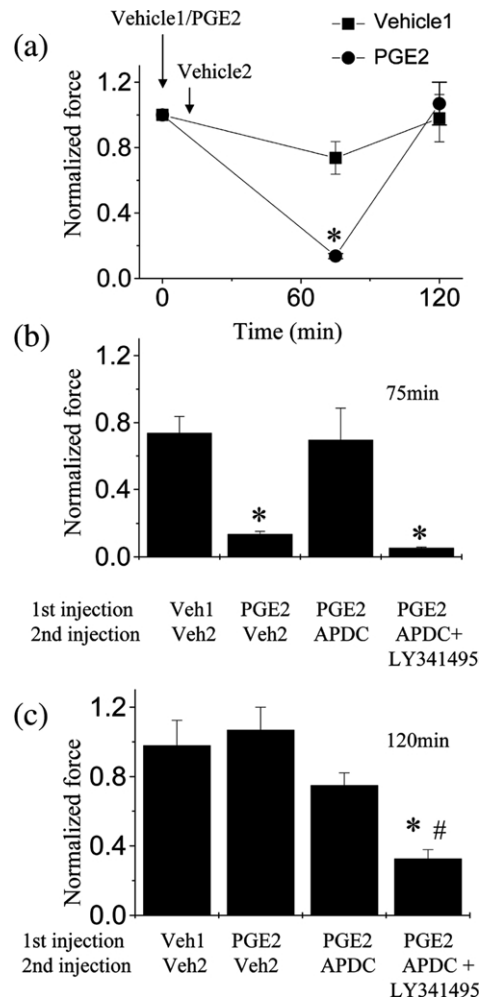


Fig. 2. Activation of group II mGluRs reduces PGE2-induced mechanical allodynia. (a) Graph shows the effects of PGE2 on mechanical thresholds. Baseline was determined as mentioned in the legend of Fig. 1. Vehicle1 (PBS + 0.1% ethanol) or PGE2 (10  $\mu$ l 0.01 mg/ml) was injected at time 0 (first injection). At 15 min, vehicle2 (PBS + 0.4 mM NaOH + 0.4 mM HCl) was injected (second injection). The injection of PGE2 induced mechanical allodynia.  $n = 7-8$ ,  $*P < 0.05$  (two-way ANOVA). (b) Mean  $\pm$  SEM of mechanical thresholds at 75 min. Vehicle2, APDC (0.4 mM, 10  $\mu$ l) or APDC + LY341495 (0.02 mM, 10  $\mu$ l) was injected at 15 min. APDC reduced PGE2-induced mechanical allodynia to the vehicle level. Co-injection of LY341495 with APDC blocked the APDC effect. \*Significantly different from Veh1, Veh2-injected mice,  $P < 0.05$  (one-way ANOVA),  $n = 7-8$ . (c) Mean  $\pm$  SEM of mechanical thresholds at 120 min. Mechanical sensitivity was not different from baseline in vehicle2 or APDC injected animals, but persisted in APDC + LY341495-injected animals. \*Significantly different from Veh1, Veh2 mice, #significantly different from PGE2, APDC-injected mice,  $P < 0.05$  (one-way ANOVA),  $n = 7-8$ .

### 3.2. Activation of peripheral group II mGluRs inhibits carageenan-induced mechanical sensitization and modulates endogenous anti-allodynia effects

The previous experiments suggest that activation of peripheral group II mGluRs inhibits PGE2-induced mechanical allodynia and that peripheral group II mGluRs may mediate endogenous anti-allodynia effects. We then tested

Table 1  
Mechanical thresholds of different treatment groups in the PGE2-induced mechanical allodynia model

Injection 1	Vehicle1	PGE2	PGE2	PGE2	PGE2
Injection 2	Vehicle2	Vehicle2	APDC	APDC + LY341495	LY341495
Baseline	1.13 ± 0.14	1.12 ± 0.17	1.01 ± 0.14	1.17 ± 0.07	1.24 ± 0.11
75 min	0.80 ± 0.14	0.14 ± 0.01	0.60 ± 0.08	0.06 ± 0.01	0.09 ± 0.02
120 min	0.94 ± 0.24	1.17 ± 0.19	0.71 ± 0.07	0.38 ± 0.07	0.14 ± 0.01

Mechanical thresholds are defined as the minimum force (in grams) required to generate a withdrawal response. Baseline was measured before any injection. Injection 1 was performed at time 0. Injection 2 was performed at 15 min. PGE2 0.01 mg/ml, APDC 400  $\mu$ M, LY341495 20  $\mu$ M. All drugs were injected in a volume of 10  $\mu$ l subcutaneously into the plantar surface of mouse hind paw.

whether peripheral group II mGluRs similarly modulate carrageenan-induced inflammation. Baseline mechanical thresholds were similar to those in the previous experiments and the thresholds significantly decreased 45 min after carrageenan injection (Fig. 4), so that most mice respond to the smallest fiber tested (0.02 g). At 55 min after carrageenan injection, we injected PBS, APDC or LY341495. Compared to PBS injection, APDC dose-dependently reduced mechanical allodynia and accelerated the recovery (Fig. 4a). In contrast, LY341495 dose-dependently prolonged allodynia (Fig. 4b). These results agree with our data in the PGE2-induced allodynia model and further suggest that endogenous activation of peripheral group II mGluRs during inflammation antagonizes mechanical allodynia.

### 3.3. Activation of peripheral group II mGluRs inhibits nociception specifically

It is possible that the inhibitory effects we observed in the previous experiments could result from an anti-inflammatory-like action of APDC, rather than a specific reduction in allodynia. To further characterize the function of group II mGluRs, we measured paw thickening following inflammation. The decreased mechanical allodynia induced by APDC (400  $\mu$ M, 10  $\mu$ l) was not accompanied by

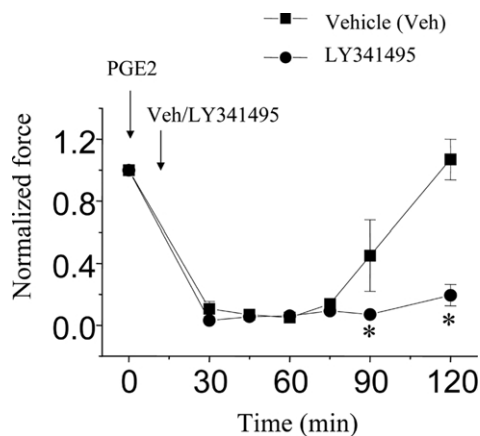


Fig. 3. Blocking peripheral group II mGluRs prolongs PGE2-induced mechanical allodynia. Graph shows the normalized mechanical thresholds measured over time. Baseline was determined as described in Fig. 1. PGE2 (10  $\mu$ l 0.01 mg/ml) was injected at 0 min. Vehicle (PBS) or LY341495 (0.02 mM, 10  $\mu$ l), a group II mGluR antagonist, was injected at 15 min.  $n = 6-8$ , \* $P < 0.05$  (two-way ANOVA).

a significant decrease in paw thickening (edema) as shown in Fig. 5. This suggests that APDC specifically inhibits inflammation-induced sensitization, rather than causing a general decrease in inflammation.

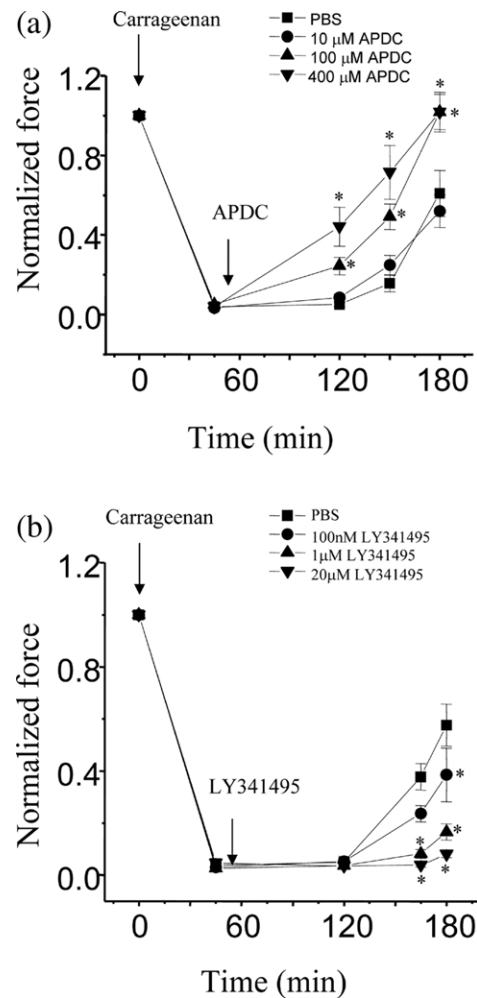


Fig. 4. Group II mGluRs modulate carrageenan-induced mechanical allodynia. (a) The injection of APDC dose-dependently reduced mechanical allodynia. Carrageenan (2%, 10  $\mu$ l) was injected at 0 min. Mechanical allodynia developed within 45 min. PBS or APDC of different concentrations (10, 100, or 400  $\mu$ M, 10  $\mu$ l) was injected at 55 min. \*Significantly different from PBS-injected mice,  $P < 0.05$ , (two-way ANOVA followed by Bonferroni post-tests)  $n = 7-9$ . (b) LY341495 dose-dependently prolonged carrageenan-induced mechanical allodynia. Carrageenan (2%, 10  $\mu$ l) was injected at 0 min. PBS, 100 nM, 1 or 20  $\mu$ M LY341495 was injected at 55 min. \*Significantly different from PBS-injected mice,  $P < 0.05$ , (two-way ANOVA followed by Bonferroni post-tests),  $n = 6-12$ .

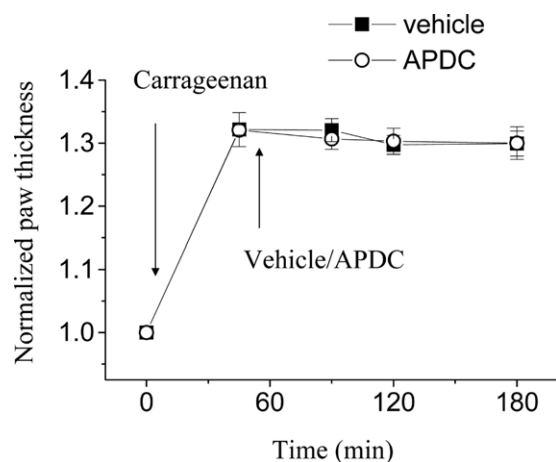


Fig. 5. Activation of group II mGluRs does not reduce carrageenan-induced paw thickening. Graph shows normalized thickness of the carrageenan-injected paw measured over time. Paw thickness before any injection was taken as the baseline to which all measurements were normalized. Carrageenan (2%, 10  $\mu$ l) was injected at 0 min. Vehicle (PBS) or APDC (400  $\mu$ M, 10  $\mu$ l) was injected at 55 min. APDC had no significant effect on the inflammation-induced increase in paw thickness (two-way ANOVA).  $n = 7-8$ .

#### 4. Discussion

Our previous studies showed that the activation of peripheral group II mGluRs could completely block PGE<sub>2</sub>-induced thermal hyperalgesia, revealing the possibility of targeting peripheral group II mGluRs in pain management (Yang and Gereau, 2002). In this study, we further tested the anti-nociceptive role of peripheral group II mGluRs in mechanical sensitization. Similar to what we found in the study of thermal sensation, subcutaneous injection of group II mGluR agonists in naïve mice did not alter mechanical sensitivity measured from 0.5 to 1.5 h after agonist injection. However, in both the carrageenan- and the PGE<sub>2</sub>-induced inflammation models, allodynia was significantly reduced by subcutaneous injection of APDC, a group II mGluR agonist, in the inflamed paw. APDC is a potent and selective agonist at group II mGluRs ( $EC_{50} = 0.4 \mu$ M), although it may activate group I or III mGluRs at higher concentrations with an  $EC_{50}$  of  $>100$  and  $>300 \mu$ M, respectively (Schoepp et al., 1999). In our experiments, 100  $\mu$ M APDC injected subcutaneously significantly reduced carrageenan-induced mechanical allodynia. Considering the dilution and diffusion in subcutaneous injection, the actual tissue concentration of APDC will likely be less than 100  $\mu$ M. Therefore, the effects we see are likely due to specific activation of group II mGluRs rather than group I or III mGluRs. Activation of group I mGluRs has been shown to cause mechanical hypersensitivity (Walker et al., 2001), also suggesting that the effects of APDC are not mediated by activation of group I mGluRs. LY341495, a potent group II mGluR antagonist, completely blocked the ability of APDC to reduce PGE<sub>2</sub>-induced mechanical allodynia, further supporting an anti-allodynia role of group II mGluRs.

The present study also investigated the role of endogenous activation of group II mGluRs in inflammation-induced mechanical allodynia. LY341495 significantly prolonged the presence of PGE<sub>2</sub>- or carrageenan-induced mechanical allodynia. In the carrageenan model, the mechanical thresholds of vehicle-injected mice recovered to half of the baseline level 3 h after carrageenan injection, while at the same time point LY341495-injected mice showed no significant recovery. These results supported the hypothesis that endogenously activated group II mGluRs can counteract the effects of other inflammatory mediators. LY341495 has a much higher potency at group II mGluRs ( $IC_{50} < 30$  nM) compared to group I or III mGluRs (Schoepp et al., 1999). However, at higher concentrations, LY341495 can also block the latter two groups of mGluRs. The effects we saw with LY341495 are unlikely a result of blocking peripheral group I mGluRs, since group I mGluRs have been shown to produce nociceptive sensitization and blocking group I mGluRs has the opposite effect of what we observed with LY341495 (Bhave et al., 2001; Walker et al., 2001). However, the  $IC_{50}$  of LY341495 for group III mGluRs ranges from 0.17 to  $>20 \mu$ M (Schoepp et al., 1999), and since group III mGluRs are also coupled to  $G_i$ , they have the potential to mediate anti-nociception effects similar to group II mGluRs. The  $IC_{50}$  for LY341495 blockade of mGluR7, which has been shown to be present in rat superior-cervical ganglion neurons (Kammermeier et al., 2000), was 1  $\mu$ M. Our data showed that 100 nM LY341495, which is unlikely to block mGluR7, significantly prolonged mechanical allodynia (Fig. 4b). However, whether other subtypes of group III mGluRs are present on peripheral sensory axons is unknown, and we cannot rule out the possibility that they may also mediate endogenous anti-nociceptive effects.

In the carrageenan-induced inflammation model, we observed that application of APDC decreased the amplitude of allodynia and shortened its duration, while LY341495 prolonged allodynia without changing the amplitude. In general, decreasing the magnitude of inflammatory pain is likely to shorten its duration and increasing the magnitude prolongs its duration. The difference we observed is probably due to the fact that carrageenan resulted in maximum allodynia measurable with our Von Frey filaments. Therefore, the pro-nociceptive effects of LY341495 were only observable at later time points when the carrageenan effects decreased. The dose of drugs applied and the choice of time for measurement could also result in a difference in observation. As shown in Fig. 4a, from the time points we have chosen, the duration of allodynia is similar in 100 and 400  $\mu$ M APDC-injected mice but the higher dose of APDC brought about a larger decrease in magnitude at the earlier time points.

The sensory fibers that conduct noxious heat are primarily C fibers, with a small percentage of A $\delta$  fibers also responding to noxious thermal stimuli. Noxious mechanical sensation, however, are conducted through both A $\delta$  fibers, most of which are heat insensitive, and C

fibers, most of which are heat sensitive (Cain et al., 2001; Campero et al., 1996). The work of Carlton et al. (2001) suggests that group II mGluRs are present on both A $\delta$  and C fibers in the skin. Our results that activation of peripheral group II mGluRs inhibits both inflammation-induced thermal hyperalgesia (Yang and Gereau, 2002) and mechanical allodynia are consistent with the localization of group II mGluRs in the periphery. However, the present results do not address the issue of whether group II mGluRs are differentially localized on different types of A $\delta$  and C fibers, which respond differently to mechanical and thermal stimuli.

We showed that APDC injected after mechanical allodynia was well-established significantly reduced allodynia and accelerated the recovery processes. Previous studies by Sharp et al. (2002) showed that pre-injection, but not post-injection, of a group II mGluR agonist (LY379268) i.p. produces anti-nociceptive effects. The reason for this difference is not clear, but may be due to different mechanisms of central and peripheral group II mGluRs in their antinociceptive effects. Differences in drugs used or lack of sufficient concentration in the periphery in the previous study could also contribute to this discrepancy.

Based on our data and previous reports, peripheral glutamate appears to be activating both groups I and II mGluRs, which produce opposing effects. During inflammation, glutamate is increased in the periphery (deGroot et al., 2000; Nordlind et al., 1993; Omote et al., 1998), where it acts at group I mGluRs to increase sensitivity to mechanical and thermal stimuli (Bhave et al., 2001; Walker et al., 2001; Zhou et al., 2001). Activation of group I mGluRs also contributes to the production of other inflammatory mediators, such as the prostaglandins (Hu et al., 2002), which also function to enhance sensitivity to touch and heat by activating the G<sub>s</sub>/cAMP/PKA pathway (Cui and Nicol, 1995; Hingtgen et al., 1995; Taiwo et al., 1989). The group II mGluRs, mGlu2 and mGlu3, are G<sub>i</sub> coupled receptors that act to oppose the prostaglandin-mediated sensitization (Yang and Gereau, 2002). It is perhaps in this context that group II mGluRs can act to reduce inflammation-induced hypersensitivity. It is possible that mGlu2/3 are not expressed in the same cells as mGlu1/5, but in the neighboring cells that are sensitized by the prostaglandins subsequent to mGlu1/5 activation. Future studies examining the co-localization of groups I and II mGluRs will be needed to address this issue.

Many iGluRs and mGluRs have been found to be expressed on primary sensory fibers (Agrawal and Evans, 1986; Bhave et al., 2001; Carlton et al., 1995, 2001; Crawford et al., 1997; Huettner, 1990; Li et al., 1997; Liu et al., 1994). Of the iGluRs, both NMDA and non-NMDA receptors, including AMPA and kainate receptors, in the periphery participate in inflammatory nociceptive sensitization (Chen et al., 1999; Jackson et al., 1995; You et al., 2002). Of the mGluRs, group I mGluRs seem to play a pro-nociceptive role while group II mGluRs play

an anti-nociceptive role. Thus the role of glutamate in the periphery during inflammation is complex and involves multiple types of receptors. It is interesting to note that the expression of group II mGluRs increases in several pain models (Boxall et al., 1998; Neto et al., 2001). Furthermore, L-acetylcarnitine, an analgesic used to treat neuropathic pain, up-regulates mGluR2 in DRG neurons (Chiechio et al., 2002). These findings suggest that the relative role of the various glutamate receptors will depend on the type and duration of injury. It will be interesting to investigate the relative role of different glutamate receptors in the induction vs. maintenance of inflammatory pain, and in short-term vs. chronic pain.

As mentioned above, subcutaneous injection of APDC when inflammation-induced mechanical allodynia was well established significantly reduced allodynia and accelerated recovery (Figs. 2 and 4). Together with the observation that group II mGluR activation did not affect basal mechanical sensation and endogenous activation of group II mGluRs modulates the recovery from mechanical allodynia, our study suggests that peripheral group II mGluRs might be a candidate for both prevention and treatment of inflammatory pain. Compared to systemic activation or intrathecal activation of group II mGluRs, which can also reduce pain sensitization, local targeting of peripheral group II mGluRs would have the advantage of reducing many possible side effects.

## Acknowledgements

The authors thank H. Hu and F. Karim for helpful comments on the manuscript. This work was supported by grants to R.G. from the NIMH (MH60230) and the NINDS (NS42595).

## References

- Agrawal SG, Evans RH. The primary afferent depolarizing action of kainate in the rat. *Br J Pharmacol* 1986;87:345–55.
- Ahlgren SC, Levine JD. Mechanical hyperalgesia in streptozotocin-diabetic rats is not sympathetically maintained. *Brain Res* 1993;616:171–5.
- Bhave G, Karim F, Carlton SM, Gereau RW. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. *Nat Neurosci* 2001;4:417–23.
- Boxall SJ, Berthele A, Laurie DJ, Sommer B, Zieglgansberger W, Urban L, Tolle TR. Enhanced expression of metabotropic glutamate receptor 3 messenger RNA in the rat spinal cord during ultraviolet irradiation induced peripheral inflammation. *Neuroscience* 1998;82:591–602.
- Cain DM, Khasabov SG, Simone DA. Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: an in vivo study. *J Neurophysiol* 2001;85:1561–74.
- Campero M, Serra J, Ochoa JL. C-polymodal nociceptors activated by noxious low temperature in human skin. *J Physiol* 1996;497(Pt 2): 565–72.
- Carlton SM, Hargett GL, Coggeshall RE. Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. *Neurosci Lett* 1995;197:25–8.

- Carlton SM, Hargrett GL, Coggeshall RE. Localization of metabotropic glutamate receptors 2/3 on primary afferent axons in the rat. *Neuroscience* 2001;105:957–69.
- Chen J, Li H, Luo C, Li Z, Zheng J. Involvement of peripheral NMDA and non-NMDA receptors in development of persistent firing of spinal wide-dynamic-range neurons induced by subcutaneous bee venom injection in the cat. *Brain Res* 1999;844:98–105.
- Chiechio S, Caricasole A, Barletta E, Storto M, Catania MV, Copani A, Verdechey M, Nicolai R, Calvani M, Melchiorri D, Nicoletti F. L-Acetylcarnitine induces analgesia by selectively upregulating mGlu2 metabotropic glutamate receptors. *Mol Pharmacol* 2002;61:989–96.
- Conn PJ, Pin J-P. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* 1997;37:205–37.
- Crawford JH, Wootton JF, Seabrook GR, Scott RH. Activation of Ca<sup>2+</sup>-dependent currents in dorsal root ganglion neurons by metabotropic glutamate receptors and cyclic ADP-ribose precursors. *J Neurophysiol* 1997;77:2573–84.
- Cui M, Nicol GD. Cyclic AMP mediates the prostaglandin E<sub>2</sub>-induced potentiation of bradykinin excitation in rat sensory neurons. *Neuroscience* 1995;66:459–66.
- Dolan S, Nolan AM. Behavioural evidence supporting a differential role for group I and II metabotropic glutamate receptors in spinal nociceptive transmission. *Neuropharmacology* 2000;39:1132–8.
- Du J, Zhou S, Coggeshall RE, Carlton SM. N-methyl-D-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. *Neuroscience* 2003;118:547–62.
- Fisher K, Lefebvre C, Coderre TJ. Antinociceptive effects following intrathecal pretreatment with selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain. *Pharmacol Biochem Behav* 2002;73:411–8.
- Gerber G, Zhong J, Youn D, Randic M. Group II and group III metabotropic glutamate receptor agonists depress synaptic transmission in the rat spinal cord dorsal horn. *Neuroscience* 2000;100:393–406.
- deGroot J, Zhou S, Carlton SM. Peripheral glutamate release in the hindpaw following low and high intensity sciatic stimulation. *NeuroReport* 2000;11:497–502.
- Hingtgen CM, Waite KJ, Vasko MR. Prostaglandins facilitate peptide release from rat sensory neurons by activating the adenosine 3',5'-cyclic monophosphate transduction cascade. *J Neurosci* 1995;15:5411–9.
- Hu HJ, Bhave G, Gereau RW. Prostaglandin and protein kinase A-dependent modulation of vanilloid receptor function by metabotropic glutamate receptor 5: potential mechanism for thermal hyperalgesia. *J Neurosci* 2002;22:7444–52.
- Huettnner JE. Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron* 1990;5:255–66.
- Jackson DL, Graff CB, Richardson JD, Hargreaves KM. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. *Eur J Pharmacol* 1995;284:321–5.
- Jia H, Rustioni A, Valtchanoff JG. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. *J Comp Neurol* 1999;410:627–42.
- Kammermeier PJ, Xiao B, Tu JC, Worley PF, Ikeda SR. Homer proteins regulate coupling of group I metabotropic glutamate receptors to N-type calcium and M-type potassium channels. *J Neurosci* 2000;20:7238–45.
- Karim F, Bhave G, Gereau RW. Metabotropic glutamate receptors on peripheral sensory neuron terminals as targets for the development of novel analgesics. *Mol Psychiatry* 2001;6:615–7.
- Khasar SG, Green PG, Levine JD. Comparison of intradermal and subcutaneous hyperalgesic effects of inflammatory mediators in the rat. *Neurosci Lett* 1993;153:215–8.
- Levine JD, Reichling DB. Peripheral mechanisms of inflammatory pain. In: Wall PD, Melzack R, editors. *Textbook of pain*. Edinburgh: Churchill Livingstone; 1999. p. 59–84.
- Li H, Ohishi H, Kinoshita A, Shigemoto R, Nomura S, Mizuno N. Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat. *Neurosci Lett* 1997;223:153–6.
- Liu H, Wang H, Sheng M, Jan LY, Jan YN, Basbaum AI. Evidence for presynaptic N-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn. *Proc Natl Acad Sci USA* 1994;91:8383–7.
- Neto FL, Schadrack J, Platzer S, Zieglgansberger W, Tolle TR, Castro-Lopes JM. Up-regulation of metabotropic glutamate receptor 3 mRNA expression in the cerebral cortex of monoarthritic rats. *J Neurosci Res* 2001;63:356–67.
- Neugebauer V, Chen PS, Willis WD. Groups II and III metabotropic glutamate receptors differentially modulate brief and prolonged nociception in primate STT cells. *J Neurophysiol* 2000;84:2998–3009.
- Nordlind K, Johansson O, Liden S, Hokfelt T. Glutamate- and aspartate-like immunoreactivities in human normal and inflamed skin. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1993;64:75–82.
- Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. *Neuroscience* 1993;53:1009–18.
- Omote K, Kawamata T, Kawamata M, Namiki A. Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. *Brain Res* 1998;787:161–4.
- Schoepp DD, Jane DE, Monn JA. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* 1999;38:1431–76.
- Sharpe EF, Kingston AE, Lodge D, Monn JA, Headley PM. Systemic pretreatment with a group II mGlu agonist, LY379268, reduces hyperalgesia in vivo. *Br J Pharmacol* 2002;135:1255–62.
- Simmons RM, Webster AA, Kalra AB, Iyengar S. Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. *Pharmacol Biochem Behav* 2002;73:419–27.
- Taiwo YO, Bjerknes LK, Goetzl EJ, Levine JD. Mediation of primary afferent peripheral hyperalgesia by the cAMP second messenger system. *Neuroscience* 1989;32:577–80.
- Walker K, Bowes M, Panesar M, Davis A, Gentry C, Kesingland A, Gasparini F, Spooren W, Stoehr N, Pagano A, Flor PJ, Vranesic I, Lingenhoehl K, Johnson EC, Varney M, Urban L, Kuhn R. Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function. I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain (In Process Citation). *Neuropharmacology* 2001;40:1–9.
- Wang C, Wang Y, Zhao Z. Peripheral NMDA and non-NMDA receptors contribute to nociception: an electrophysiological study. *Brain Res Bull* 2000;52:31–4.
- Yang D, Gereau RW. Peripheral group II metabotropic glutamate receptors (mGluR2/3) regulate prostaglandin E<sub>2</sub>-mediated sensitization of capsaicin responses and thermal nociception. *J Neurosci* 2002;22:6388–93.
- You HJ, Chen J, Morch CD, Arendt-Nielsen L. Differential effect of peripheral glutamate (NMDA, non-NMDA) receptor antagonists on bee venom-induced spontaneous nociception and sensitization. *Brain Res Bull* 2002;58:561–7.
- Zhou S, Komak S, Du J, Carlton SM. Metabotropic glutamate 1alpha receptors on peripheral primary afferent fibers: their role in nociception. *Brain Res* 2001;913:18–26.