International Journal of Oral Biology, Vol. 33, No. 3, September 30 2008, p. 97~103

International Journal of Oral Biology

Peripheral metabotropic glutamate receptors differentially modulate mustard oil-induced craniofacial muscle pain in lightly anesthetized rats

Min K. Lee, Gwi Y. Yang, and Dong K. Ahn*

Department of Oral Physiology and Institution of BrainKorea21, School of Dentistry, Kyungpook National University, Daegu, Korea

(Received June 14, 2008 ; Revised June 18, 2008 ; Accepted June 20, 2008)

The present study investigated the role of peripheral group I, II, and III metabotropic glutamate receptors (mGluRs) in mustard oil (MO)-induced nociceptive response in the masseter muscles of lightly anesthetized rats. Experiments were carried out on male Sprague-Dawley rats weighing 300-350 gm. After initial anesthesia with sodium pentobarbital (40 mg/kg, i.p.), one femoral vein was cannulated and connected to an infusion pump for intravenous infusion of sodium pentobarbital. The rate of infusion was adjusted to provide a constant level of anesthesia. MO (30 μL) was injected into the mid-region of the left masseter muscle via a 30-gauge needle over 10 seconds. After 30 mL injection of 5, 10, 15, or 20% MO into the masseter muscle, total number of hindpaw-shaking behavior was monitored. Intramuscular administration of MO significantly produced hindpaw-shaking behavior in a dose-dependent manner, as compared with the vehicle (mineral oil)-treated group. Intramuscular pretreatment with 10 or 100 ng DHPG, a group I mGluRs agonist, enhanced MO-induced hindpaw-shaking behavior, while APDC (20 or 200 μg), a group II mGluRs agonist, or L-AP4 (2 μg), a group III mGluRs agonist, significantly reduced MO-induced nociceptive behavior. The antinociception, produced by group II or III mGluRs agonists, was abolished by pretreatment with LY341495, a group II mGluRs antagonist, or CPPG, a group III mGluRs antagonist, res-pectively. Based on these observations, peripheral mGluRs differentially modulated MO-induced nociceptive behavior response in the craniofacial muscle pain and peripheral group II and III mGluRs agonists could be used in treatment of craniofacial muscle nociception.

*Corresponding author: Dong-Kuk, Ahn, DDS, PhD. Department of Oral Physiology, School of Dentistry, Kyungpook National University, 188-1 Sam Deok 2 ga, Chung-gu, Daegu (700-412), Korea. Tel.: 82-53-660-6840; Fax.: 82-53-421-4077; E-mail: dkahn@knu.ac.kr

Key words: mGluRs, glutamate, muscle pain, antinociception

Introduction

Excitatory amino acids, primarily glutamate, play a significant role in nociceptive transmission as an excitatory neurotransmitter in the spinal cord (Watkins and Evans, 1981) and the trigeminal subnucleus caudalis (Clements *et al*., 1991). Glutamate activates not only ionotropic glutamate receptors but also metabotropic glutamate receptors (mGluRs) that coupled to G-proteins (Conn and Pin, 1997; Schoepp *et al*., 1999). Eight mGluR subtypes are classified into three groups based on their sequence homology, signal transduction mechanisms and pharmacological profile (Pin and Duvoisin, 1995). Group I mGluRs (mGluR 1 and 5) are positively coupled to phospolipase C, while group II (mGluR 2 and 3) and group III (mGluR 4, 6, 7, and 8) mGluRs are negatively coupled to adenylate cyclase (Ohishi *et al*., 1993a,b; Schoepp and Conn, 1993).

Accumulating evidence suggests that spinal group I mGluRs play a pivotal role in acute nociception, inflammatory pain and hyperalgesia. Metabotropic glutamate receptors (mGluR5) or binding sites have been demonstrated to be present in the dorsal horn of the spinal cord (Jia *et al*., 1999) and the spinal trigeminal nucleus (Tallaksen-Greene *et al*., 1992), particularly in the superficial laminae (laminae I and II). The second phase of nociceptive behavior induced by formalin was reported to be enhanced by DHPG, an mGluR1/5 agonist (Fisher and Coderre, 1996). Group II and III mGluRs also have been detected in the dorsal horn of the spinal cord (Jia *et al*., 1999; Taylor *et al*., 2003). Intraperitoneal injection of LY354740, LY379268 or LY389795, selective group II mGluR 2/3 agonists,

attenuated the second phase of the formalin-induced paw-licking behavior in rats (Simmons *et al*., 2002). Intrathecal administration of L-AP4, a group III mGluR agonist, also attenuated allodynia and neuronal responses in a model of neuropathic pain (Chen *et al*., 2005).

Previous studies demonstrated that peripheral mGluRs play an important role in the nociceptive transmission. Intraplantar administration of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), an mGluR5 antagonist, significantly reduced Freund's complete adjuvant-induced inflammatory hyperalgesia (Walker *et al*., 2001a, b). Group II or group III is expressed myelinated and unmyelinated axons in the digital nerves or in the small diameter primary afferents, respectively (Carlton *et al*., 2001). Recent behavioral studies have shown that activation of group I, II and III mGluRs is involved in orofacial nociceptive processing. Subcutaneous administration of CPCCOEt or (S)-(+)-α-amino-4-carboxyl-2-ethylbenzeneacetic acid (LY367385), a mGluR1 antagonist, as well as MPEP or 2-Methyl-6-(2-phenylethenyl)pyridine (SIB1893), a mGluR5 antagonist, abolished IL-1b-induced mechanical allodynia (Ahn *et al*., 2005). Similarly, subcutaneous administration of APDC or (2S ,2'R, 3'R)-2-(2', 3'-Dicarboxycyclopropyl) glycine (DCG IV), a group II mGluR agonist, blocked IL-1b-induced mechanical allodynia (Ahn *et al*., 2005). These results suggest that peripheral mGluRs play an important role in the peripheral nociception in the orofacial area. However, the involvement of peripheral mGluRs in the processing of craniofacial muscle pain has not been studied.

The present study was designed to investigate role of peripheral mGluRs in the nociceptive processing from craniofacial muscle. To achieve this purpose, we examined effects of intramuscular administration of mGluRs on the hindpaw-shaking nociceptive behavior produced by the intramuscular injection of MO into the masseter muscles in lightly anesthetized rats.

Materials and Methods

Animals

All procedures involving the use of animals were approved by the Institutional Care and Use Committee of the School of Dentistry, Kyungpook National University and carried out in accordance with the ethical guidelines for the investigation of experimental pain in animals by the International Association for the Study of Pain. Experiments were carried out on 141 male Sprague-Dawley rats weighing between 300~350 gm. They were maintained in a temperature-controlled room (23 ± 1°C) with a 12/12-hour light/dark cycle. In each experiment, the experimenter was blind to the treatment groups.

General procedures

Behavioral assessment of craniofacial muscle pain was performed in lightly anesthetized rat model as previously described (Lee *et al*., 2006; Ro *et al*., 2003). After initial anesthesia with sodium pentobarbital (40 mg/kg, i.p.), one femoral vein was cannulated and connected to an infusion pump (Pump 22, Harvard Apparatus, Massachusetts, U.S.A.) for intravenous infusion of sodium pentobarbital. The rate of infusion was adjusted to provide a constant level of anesthesia (3-5 mg/h). Rectal temperature was monitored and maintained within normal physiological limits during experiments. A level of "light" anesthesia was determined by providing a noxious pinch to the tail or the hindpaw with a serrated forceps as previously described (Lee *et al*., 2006; Ro *et al*., 2003). Animals typically responded to the noxious pinch of the tail with an abdominal contraction and to the noxious pinch of a hindpaw with a withdrawal reflex within 30 min after the initial anesthesia. At this point, infusion rates were adjusted and experiments were continued only after the animals showed reliable reflex responses to every noxious pinch as previously described (Lee *et al*., 2006; Ro *et al*., 2003).

Evaluation of craniofacial muscle pain

The present study examined ipsilateral hindpaw-shaking behavior evoked by administration of MO into the masseter muscle as muscle pain scores. Intramuscular injection of 5, 10, 15, or 20% of MO (30 μL) was made into the mid-region of the left masseter muscle via a 30-gauge cannula. To minimize the effects of injection of the cannula into the muscle on the hindpaw shaking behavior, a cannula was inserted into the masseter muscle 10 min prior to injection of MO. The injection cannula consisted of a 30-gauge needle connected to a PE10 tube and a Hamilton syringe. The MO was manually infused through the injection cannula over 10 seconds. Intramuscular injection of MO produced ipsilateral hindpaw-shaking behavioral response. The MO-induced hindpaw-shaking behavior was quantified by counting the total number of shaking behavior for four minutes after intramuscular injection of MO. The magnitude of the behavioral response was highly correlated with the concentration of MO. All counts were made by one experimenter to maintain the consistency of counting. Mineral oil was used as the control injection for MO.

Role of peripheral mGluRs in nociceptive behavior in the craniofacial muscle pain

The present experiment was examined that the effects of peripheral mGluRs on hindpaw-shaking behavioral responses produced by intramuscular injection of MO. DHPG (0.1 ng, 1 ng, 10 ng, 100 ng/ 50 μL), a selective group I mGluRs agonist, APDC (0.2 μg, 2 μg, 20 μg, 200 μg/ 50 μL), a group II mGluRs agonist, L-AP4 (0.2 μg, 2 μg, 20 μg, 100 μg/ 50 μL), a group III mGluRs agonist, were injected into the masseter muscle 20 min prior to 20% of MO injection. LY341495 (20 ng/ 50 μL), a group II mGluRs antagonist, and CPPG (1 mg/ 50 μL), a group III mGluRs

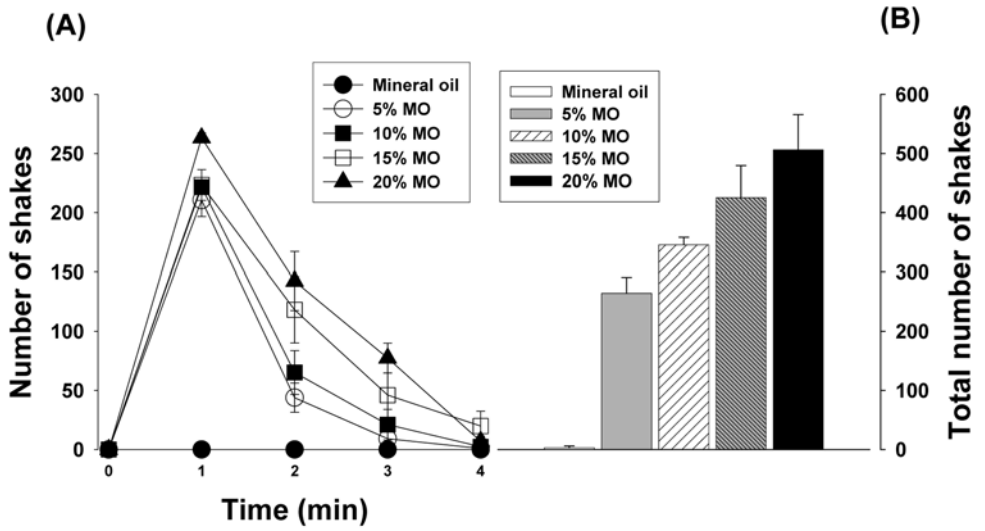

Fig. 1. Time course of MO-induced ipsilateral hindpaw-shaking behavioral response (A) and total number of shakes (B) in the masseter muscle. Animals received a 30 μL intra-muscular injection of 5, 10, 15, 20% MO into the masseter muscle. The number of hindpaw-shaking behavior was measured for 4 minutes. There were 6 animals in each group.

antagonist, were injected into the masseter muscle 10 min prior to injection of mGluRs agonist, respectively. After intramuscular injection of MO, hindpaw-shaking behavioral responses were measured. NaOH vehicle (50 μL) was used as the injection of control for group II and III mGluRs agonists and antagonists, and saline (50 μL) was used as the injection of control for group I mGluRs agonist.

Chemicals

(S)-3,5-dihydroxyphenylglycine (DHPG), APDC, LY341495, L-AP4, and (RS)-a-cyclopropyl-4-phosphonophenylglycine (CPPG), were obtained from Tocris. MO was purchased from Sigma. APDC, LY341495, L-AP4 and CPPG were made in NaOH vehicle. DHPG was dissolved in normal saline. MO was diluted with mineral oil.

Data analysis

Differences between groups were compared using analysis of variance (ANOVA), followed by LSD post hoc analysis. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance. All data are presented as mean ± SEM.

Results

The present study demonstrated that intramuscular injection of MO produced nociceptive hid-paw scratching behavior. Animals maintained under light anesthesia showed no significant spontaneous hindpaw-shaking behavioral responses prior to MO injection. Microinjection of 30 μL MO (5, 10, 15 or 20%) into the masseter muscle produced ipsilateral hindpaw-shaking behavioral responses appeared to be directed to the injected site in a dose-dependent manner. The hindpaw-shaking behavior lasted for several minutes with peak number of shakes occurring within 1 minute after intramuscular injection (Fig. 1A). After injection of 5, 10, 15, or 20% MO into the masseter muscle, the counts of shaking behavior were 264 ± 26, 345 ± 12, 425 ± 54, or 506 ± 59 number of scratches, respectively, and it was significantly higher in the MO-treated group than the vehicle (mineral oil)-treated group ($p < 0.05$; Fig. 1B). Administration of 30 μL mineral oil did not evoke the hindpaw-shaking behavior.

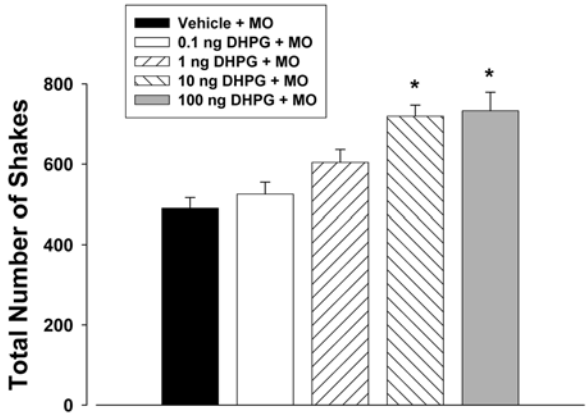

Fig. 2. The effects of intramuscular pretreatment with DHPG, a non-selective group I mGluRs agonist, on MO-induced hindpaw-shaking behavior. DHPG was administrated intramuscularly 20 min prior to the injection of 20% MO in to masseter muscle. Intramuscular pretreatment with 10 or 100 ng DHPG significantly increased MO-induced hindpaw-shaking behavior compared with the vehicle-treated group ($p<0.05$). There were 6 animals in each group. *p <0.05, vs. vehicle + MO-treated group.

The effects of DHPG, a selective group I mGluRs agonist, on hindpaw-shaking behavioral responses produced by MO

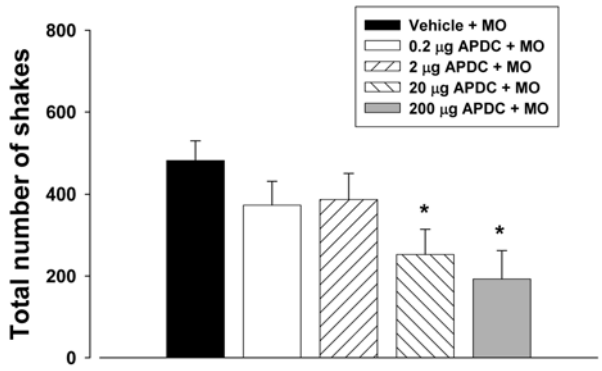

Fig. 3. The effects of intramuscular pretreatment with APDC, a group II mGluRs agonist, on MO-induced hindpaw-shaking behavior. APDC was administrated intramuscularly 20 min prior to the injection of 20% MO in to masseter muscle. Intramuscular pretreatment with 20 or 200 μg of APDC significantly attenuated MO-induced hindpaw-shaking behavior compared with the vehicle-treated group ($p<0.05$). There were 6 animals in each group. *p <0.05, vs. vehicle + MO-treated group.

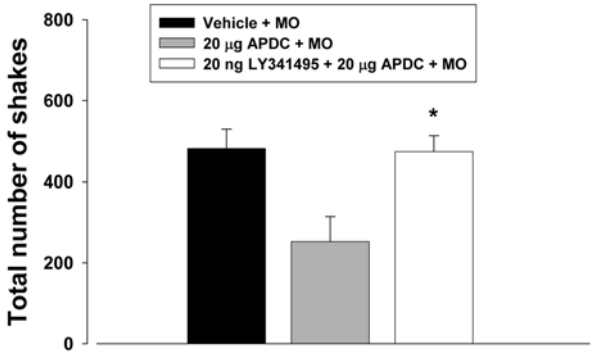

Fig. 4. The effects of intramuscular pretreatment with LY341495, a group II mGluRs antagonist, on APDC-induced antinociception. LY341495 was administrated intramuscularly 10 min prior to the injection of APDC into the masseter muscle. Intramuscular pretreatment with 20 ng of LY341495 significantly recovered hindpaw-shaking behavior ($p<0.05$). There were 6 animals in each group. *p <0.05, *vs*. APDC + MO-treated group.

injection are illustrated in Fig. 2. Neither intramuscular administration of vehicle nor 0.1 or 1 ng of DHPG affected MO-induced hindpaw-shaking behavioral responses. However, intramuscular pretreatment with 10 or 100 ng DHPG significantly increased MO-induced hindpaw-shaking behavior, compared with the vehicle-treated group ($p < 0.05$).

Fig. 3 illustrates the effect of the intramuscular injection of APDC, a group II mGluRs agonist, on MO-induced ipsilateral hindpaw-shaking behavioral responses. Intramuscular administration of vehicle, 0.2 or 2 μg did not affect MO-induced hind-paw scratching behavior. However, intramuscular pretreatment with 20 or 200 μg APDC significantly attenuated MO-induced hindpaw-shaking

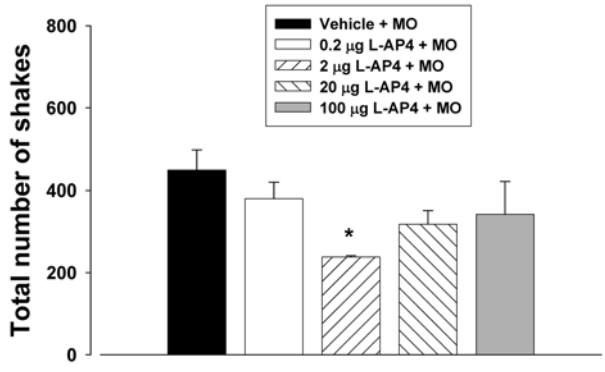

Fig. 5. The effects of intramuscular pretreatment with L-AP4, a group III mGluRs agonist, on MO-induced hindpaw-shaking behavior. L-AP4 was administrated intramuscularly 20 min prior to the injection of 20% MO in to masseter muscle. Intramuscular pretreatment with 2 μg of L-AP4 significantly attenuated MO-induced hindpaw-shaking behavior compared with the vehicle-treated group ($p<0.05$). There were 6 animals in each group. *p <0.05, vs. vehicle + MO-treated group.

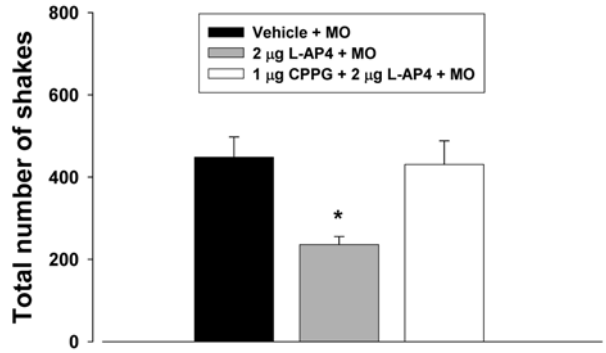

Fig. 6. The effects of intramuscular pretreatment with CPPG, a group III mGluRs antagonist, on L-AP4- induced antinociception. CPPG was administrated intramuscularly 10 min prior to the injection of L-AP4. Intramuscular pretreatment with 1 mg of CPPG significantly blocked L-AP4-induced antinociception ($p<0.05$). There were 6 animals in each group. *p <0.05, vs. L-AP4 + MO-treated group.

behavior response, compared with the vehicle-treated group ($p < 0.05$). The decreased hind-paw scratching behavior produced by APDC was blocked by intramuscular pretreatment with 20 ng of LY341495, a group II mGluR1 antagonist ($p < 0.05$; Fig. 4).

Fig 5 illustrates effects of the intramuscular injection of L-AP4, a group III mGluRs agonist, on MO-induced ipsilateral hindpaw-shaking behavioral responses. Intramusclular injection of 2 μg of L-AP4, a group III agonist, significantly attenuated MO-induced hindpaw-shaking behavior, as compared with the vehicle-treated group ($p < 0.05$; Fig. 5). The antinociceptive action produced by L-AP4 was blocked by intramuscular pretreatment with 1 mg CPPG, a group III mGluR antagonist, prior to the injection of 2 μg L-AP4 into the masseter muscle ($p < 0.05$; Fig. 6).

Discussion

The previous studies have introduced a number of experimental animal models for muscle pain (Kehl *et al*., 2000; Loram *et al*., 2007; Sluka, 2002). Injection of carrageenan in fore- or hindlimb triceps muscle produced nociceptive response, which is represented by the reduction of grip force (Kehl *et al*., 2000). Intra-gastrocneminus injection of capsaicin also produced persistent mechanical allodynia and heat hyperalgesia (Sluka, 2002). However, there are limited experimental animal models for craniofacial muscle pain due to structural complexity of craniofacial area and difficulty of behavioral measurement for craniofacial pain. Since the introduction of intramuscular injection of hypertonic saline for the craniofacial muscle pain model (Kellgren, 1938), the hypertonic saline model have been widely used to study experimental craniofacial muscle pain. Recently, a new behavioral assessment of craniofacial muscle pain in the lightly anesthetized or awaken rats was introduced by Ro group (Ro *et al*., 2003; Ro, 2005). An immediately and intense ipsilateral hindpaw-shaking behavioral responses occurring after intramuscular injection of allyl isothiocyanate (mustard oil, MO), which is C-fiber irritant, was assessed (Ro *et al*., 2003).

The present study also demonstrated that intramuscular application of MO into the masseter muscle produced consistent and intense ipsilateral hindpaw-shaking behavioral response in lightly anesthetized rats. Intramuscular injection of MO produced a characteristic ipsilateral hindpaw-shaking, which mimics the pain-induced grooming behavior in intact rats. In addition, the hindpaw-shaking behavioral responses appeared to be directed to the injected site as an attempt to rub or scratch the affected region. These results indicate that evaluation of MO-induced ipsilateral hindpaw-shaking behavior is a valid measurement of the craniofacial muscle pain.

The present study examined whether peripheral group I, II, and III mGluRs mediate the hindpaw-shaking behavior produced by MO injection into the masseter muscle. DHPG, APDC, and L-AP4 were intramuscularly administered 20 min prior to the injection of 20% MO into the masseter muscle. Intramuscular pretreatment with DHPG significantly increased MO-induced hindpaw-shaking behavior, while intramuscular pretreatment with APDC and L-AP4 significantly attenuated MO-induced hindpaw-shaking behavior. These results suggest that although peripheral mGluRs play an important role in the nociceptive transmission in craniofacial area, they differentially modulate craniofacial muscle pain.

Involvement of peripheral group I mGluRs in the nociceptive transmission has been introduced by previous studies. Bhave *et al*. (2001) demonstrated that peripheral administration of DHPG produced thermal hypersensitivity and DHPG-induced thermal hypersensitivity was reduced by pretreatment with MPEP, mGluR5 antagonist, and LY367385, mGluR1 antagonist. The injection of DHPG into the naive rat hindpaw also produced mechanical and inflammatory hyperalgesia, and DHPG-induced nociceptive responses were inhibited by pretreatment with MPEP, mGluR5 antagonist (Walker *et al*., 2001a, b). Recently, Lee *et al*. (2006) also demonstrated the involvement of peripheral group I mGluRs in MO-induced nociceptive behavior and inflammation in the masseter muscles. Intramuscular pretreatment with MCPG, a non-selective group I and II mGluRs antagonist, produced nociceptive scratching behavior and inflammation in the master muscle. Intramuscular pretreatment with MPEP significantly reduced MO-induced hindpaw-shaking behavior. Accordingly, these results taken together with present data suggest that peripheral group I mGluRs play an important role in the processing of craniofacial muscle pain.

The present study demonstrated that intramuscular administration of group II and III mGluRs significantly attenuated MO-induced nociceptive scratching behavior. These results are consistent with the previous studies. Intrathecal administration of (+/−)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD) or (2S,1S, 2S)-2-(carboxycyclopropyl)glycine (L-CCG-I), group II mGluRs agonists, significantly increased mechanical withdrawal threshold (Dolan and Nolan, 2000) and intrathecal administration of APDC was effectively decreased the development of mechanical and cold hypersensitivity associated with chronic constriction injury in rats (Fisher *et al*., 2002). Similarly, intrathecal administration of L-AP4, a group III mGluRs agonist, decreased nociceptive scores in the formalin test (Fisher and Coderre, 1996) and inhibited capsaicin-induced central sensitization (Neugebauer *et al*., 2000). While a number of studies have focused on nociceptive transmission in CNS, less is known about the specific role of group II and group III mGluRs in the nociceptive precessing in the craniofacial area. The present study demonstrated that intramuscular pretreatment with APDC and L-AP4 significantly attenuated MO-induced hindpaw-shaking behavior. The antinociceptive action produced by peripheral administration APDC or L-AP4, group II or III mGluRs agonists, was blocked by intramuscular injection of LY341495 or CPPG, group II or III mGluRs antagonists, respectively. These results suggest that peripheral administration of group II or III mGluR may attenuate in MO-induced nociceptive behavior in the craniofacial muscle.

Interestingly, group I mGluRs, found both pre- and postsynaptically, may increase neurotransmitter release (Pin and Duvoisin, 1995) and resulted in enhancement of neurnal excitability (Neugebauer, 2002). On the contrary to group I mGluRs, group II and III mGluRs are found primarily presynaptically (Lujan *et al*., 1996; Neki *et al*., 1996; Shigemoto *et al*., 1997), where they inhibit neurotransmission (Gereau and Conn, 1995; Macek *et al*.,

1996; Vignes *et al*., 1995). Moreover, activation of group I mGluRs in persistent nociception lead to the activation of phosphoinostide hydrolysis, which results in increased intracellular Ca2+ concentration and the production of protein kinase C (Conn and Pin, 1997). However, activation of group II and III mGluRs may be effective at alleviating persistent nociception, following inhibition of cyclic adenosine monophosphate (Cozzi *et al*., 1997; East *et al*., 1995; Battaglia *et al*., 1997). Although these results showed underlying cellular or molecular mechanisms of mGluRs, the exact underlying cellular or molecular mechanisms of peripheral mGluRs in craniofacial muscle need to be further study.

In summary, intramuscular injection of MO produced nociceptive scratching behavioral response. Intramuscular injection of DHPG, group I mGluRs agonist, enhanced MO-induced craniofacial muscle pain. However, intramuscular injection of APDC or L-AP4, group II or III mGluRs agonists, reduced MO-induced craniofacial muscle pain. The antinociception, produced by APDC or L-AP4, was abolished by pretreatment with LY341495 or CPPG, group II or III mGluRs antagonist, respectively. Based on these observations, peripheral mGluRs differentially modulated MO-induced nociceptive behavior response in the craniofacial muscle pain and blockade of peripheral group II or III mGluRs could be used in treatment of craniofacial muscle nociception.

Acknowledgement

This work was supported by grants (R01-2006-000-10488-0 to DKA) from the Basic Research Program of the Korea Science & Engineering Foundation.

References

Ahn DK, Kim KH, Jung CY, Choi HS, Lim EJ, Youn DH, Bae YC. Role of peripheral group I and II metabotropic glutamate receptors in IL-1b-induced mechanical allodynia in the orofacial area of conscious rats. Pain 2005;118:53-60.

Battaglia G, Monn JA, Schoepp DD. In vivo inhibition of veratridine-evoked release of striatal excitatory amino acids by the group II metabotropic glutamate receptor agonist LY354740 in rats. Neurosci Lett. 1997;229:161-4.

Bhave G, Karim F, Carlton SM, Gereau IV RW. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat Neurosci. 2001;4:417-23.

Carlton SM, Hargett GL, Coggeshall RE. Localization of metabotropic glutamate receptors 2/3 on primary afferent axons in the rat. Neuroscience 2001;105:957-69.

Chen SR, Pan HL. Distinct roles of group III metabotropic glutamate receptors in control of nociception and dorsal horn neurons in normal and nerve-injured rats. J Pharmacol Exp Ther. 2005;312:120-6.

Clements JR, Magnusson KR, Hurtman J, Beitz AJ. Rat tooth pulp projections to spinal trigeminal subnucleus caudalis are glutamate-like immunoreactivity. J Com Neurol. 1991;309:281-8.

Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Ann Rev Pharmacol Toxicol. 1997;37:205-37.

Cozzi A, Attucci S, Peruginelli F, Marinozzi M, Luneia R, Pellicciari R, Moroni F. Type 2 metabotropic glutamate (mGlu) receptors tonically inhibit transmitter release in rat caudate nucleus: in vivo studies with (2S,1'S,2'S,3'R)-2-(2'-carboxy-3'-phenylcyclopropyl)glycine, a new potent and selective antagonist. Eur J Neurosci . 1997;9:1350-5.

Dolan S, Nolan AM. Behavioral evidence supporting a differential role for group I and II metabotropic glutamate receptors in spinal nociceptive transmission. Neuropharmacology 2000;39:1132-8.

East SJ, Hill MP, Brotchie JM. Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synaptosomes. Eur J Pharmacol. 1995;277:117-21.

Fisher K, Coderre TJ. The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. Pain 1996;68:255-63.

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.

Gereau IV RW, Conn PJ. Multiple presynaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. J Neurosci. 1995;15:6879-89.

Jia H, Rustioni A, Valtschanoff JG. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. J Comp Neurol. 1999;410:627-42.

Kehl LJ, Trempe TM, Hargreaves KM. A new animal model for assessing mechanisms and management of muscle hyperalgesia. Pain 2000;85:333-43.

Kellgren JH. Observations on referred pain arising from muscle. Clin Sci. 1938;3:175-90.

Lee HJ, Choi HS, Ju JS, Bae YC, Kim SK, Yoon YW, Ahn DK. Peropheral mGluR5 antagonist attenuated craniofacial muscle pain and inflammation but not mGluR1 antagonist in lightly anesthetized rats. Brain Res Bull. 2006;70:378-85.

Loram LC, Fuller A, Fick FG, Cartmell T, Poole S, Mitchell D. Cytokine Profiles During Carrageenan-Induced Inflammatory Hyperalgesia in Rat Muscle and Hind Paw. J Pain 2007;8(2):127-36.

Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P. Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci. 1996;8:1488-500.

Macek TA, Winder DG, Gereau IV RW, Ladd CO, Conn PJ. Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. J Neurophysiol. 1996;76:3798-806.

Neki A, Ohishi H, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N. Pre- and postsynaptic localization of a metabotropic glutamate receptor, mGluR2, in the rat brain: an

immunohistochemical study with a monoclonal antibody. Neurosci Lett. 1996;202:197-200.

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.

Neugebauer V. Metabotropic glutamate receptors--important modulators of nociception and pain behavior. Pain 2002;98:1-8.

Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. Neuroscience 1993a;53: 1009-18.

Ohishi H, Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study. J Comp Neurol. 1993b;335:252-66.

Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995;34:1-26.

Ro JY, Capra N, Masri R. Development of a behavioral assessment of craniofacial muscle pain in lightly anesthetized rats. Pain 2003;104:179-85.

Ro JY. Bite force measurement in awake rats: a behavioral model for persistent orofacial muscle pain and hyperalgesia. J Orofac Pain 2005;19:159-67.

Schoepp DD, Conn PJ. Metabotropic glutamate receptors in brain function and pathology. Trensd Pharmacol Sci. 1993;14:13-20.

Schoepp DD, Jane DE, Monn JA. Pharmacologyical agents acting at subtypes of metabotropic glutamate receptors. Neuropharmacology 1999;38:1431-76.

Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S, Mizuno N. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci. 1997;17:7503-22.

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.

Sluka KA. Stimulation of deep somatic tissue with capsaicin produces long-lasting mechanical allodynia and heat hypoalgesia that depends on early activation of the cAMP pathway. J Neurosci. 2002;22:5687-93.

Tallaksen-Greene SJ, Young AB, Penney JB, Beitz AJ. Excitatory amino acid binding sites in the trigeminal principal sensory and spinal trigeminal nuclei of the rat. Neurosci Lett. 1992;141:79-83.

Taylor DL, Diemel LT, Pocock JM. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. J Neurosci. 2003;23:2150-60.

Vignes M, Clarke VR, Davies CH, Chambers A, Jane DE, Watkins JC, Collingridge GL. Pharmacological evidence for an involvement of group II and group III mGluRs in the presynaptic regulation of excitatory synaptic responses in the CA1 region of rat hippocampal slices. Neuro−pharmacology 1995;34:973-82.

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 (mGluR5) and nociceptive function. I. Selective blockade of mGluR5 receptors in model of acute, persistent and chronic pain. Neuropharmacology 2001a;40:1-9.

Walker K, Reeve A, Bowes M, Winter J, Wotherspoon G, Davis A, Schmid P, Gasparini F, Kuhn R, Urban L. mGluR5 receptors and nociceptive function II. mGluR5 receptors functionally expressed on peripheral sensory neurons mediate inflammatory hyperalgesia. Neuropharmacology 2001b;40:10-19.

Watkins JC, Evans RH. Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol. 1981;21:165-20.