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Peripheral mGluR5 antagonist attenuated craniofacial muscle pain and inflammation but not mGluR1 antagonist in lightly anesthetized rats

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Abstract

The present study investigated the role of peripheral group I metabotropic glutamate receptors (mGluRs) in MO-induced nociceptive behaviour and inflammation in the masseter muscles of lightly anesthetized rats. Experiments were carried out on male Sprague–Dawley rats weighing 300–400 g. 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. Mustard oil (MO, 30 μl) was injected into the mid-region of the left masseter muscle via a 30-gauge needle over 10 s. After 30 μl injection of 5, 10, 15, or 20% MO into the masseter muscle, the total number of hindpaw shaking behavior and extravasated Evans' blue dye concentration in the masseter muscle were significantly higher in the MO-treated group in a dose-dependent manner compared with the vehicle (mineral oil)-treated group. Intramuscular pretreatment with 3 or 5% lidocaine reduced MO-induced hindpaw shaking behaviour and increases in extravasated Evans' blue dye concentration. Intramuscular pretreatment with 5 mM MCPG, non-selective group I/II mGluR antagonist, or MPEP, a selective group I mGluR5 antagonist, produced a significant attenuation of MO-induced hindpaw shaking behaviour and increases in extravasated Evans' blue dye concentration in the masseter muscle while LY367385, a selective group I mGluR1 antagonist, did not affect MO-induced nociceptive behaviour and inflammation in the masseter muscle. These results indicate that peripheral mGluR5 plays important role in mediating MO-induced nociceptive behaviour and inflammation in the craniofacial muscle.

Keywords: Antinociception; Muscle inflammation; Muscle pain; Nociceptive behaviour; Peripheral mGluR

1. Introduction

Deep craniofacial tissues such as masseter muscle represent common sites for acute and chronic pain [7]. Temporomandibular pain dysfunction disorders typically produce masticatory muscle pain, temporomandibular joint sounds, and neuromuscular changes reflected in limitation of jaw movement [43,48]. Pain from deep tissues is diffuse, aching and difficult to localize, whereas that from cutaneous tissues is typically sharp and easy to localize [35,44]. Application of inflammatory irritant to the temporomandibular joint increased jaw and neck muscle activity in rats [57] and human [58]. Since a muscle pain model was introduced with intramuscular injection of hypertonic saline [29], intramuscular injection has widely used in experimental muscle pain models. Injection of a single bolus of hypertonic saline in limb muscle [21,22], neck muscle [2], lower back [3], or jaw muscles [28,46] produced a local area of transient pain similar in quality and intensity to clinical myalgia. Other exogenous agents including acid saline, capsaicin, carrageenan, mustard oil (MO), and complete Freund's adjuvant also produce intense pain and inflammatory responses in muscle [23,34,45].

It is well known that excitatory amino acid receptors are present on the peripheral ends of small diameter primary afferents [8,14]. A pronounced and sustained level of glutamate released into the peripheral tissue following injury and inflam-

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mation suggests a potential involvement of excitatory amino acid and their receptors in peripheral nociceptive mechanism [30,36]. Glutamate injected into the mouse paw produced rapidly dose-related pain responses and edema formation, suggesting that peripheral excitatory amino acid receptors play an important role in nociception as well as in inflammation [30]. Furthermore, injection of glutamate into the masseter muscle excited and sensitized rat masseter muscle afferent fibers through activation of peripheral excitatory amino acid receptors in anesthetized rats [6] and produced increases in jaw and neck muscle activity and mechanical allodynia in human subjects [47,49,50]. These results suggest that peripheral glutamate plays an important role in nociception of craniofacial muscle.

Glutamate at the inflammation site modulates nociception by directly activating its ionotropic receptors [1,17]. The contribution of peripheral ionotropic glutamate receptors in craniofacial muscle nociception and inflammation has been suggested. Intravenous pretreatment with MK-801 reduced MO-induced increases in the electromyography activity of masseter and digastric muscle, and Evans Blue plasma extravation in rats [57] and reduced the MO-induced nocifensive behaviour in lightly anesthetized rats [41]. Bhave et al. [5] reported for the first time that the exogenous activation of peripheral metanotropic glutamate receptor (mGluR) 1/5 affects normal nociception in mice. A subcutaneous injection of a group I agonist (mGluR 1/5) produced long lasting thermal hypersensitivity, which was reduced by subcutaneous injections of an mGluR 1/5 antagonist [5]. Intraplantar injection of DHPG, a group I mGluR agonist, increased thermal sensitivity by enhancing vanilloid (capsaicin) receptor function in mice [ ] and intraplantar injection of AIDA or MPEP, selective mGluR1 or mGluR5 antagonist, significantly attenuated mechanical hyperalgesia or inflammatory pain [59]. These results indicate that peripheral glutamate at the inflammation site modulates nociceptive processing by directly activating its metabotropic receptors on primary afferent nociceptors. Although the participation of peripheral metabotropic glutamate receptors in the processing of pain and inflammation has been demonstrated, the contributions of peripheral mGluR in craniofacial muscle pain and inflammation have not been investigated. The present study investigated the role of peripheral group I mGluRs in MO-induced nociceptive behaviour and inflammation in the masseter muscles of lightly anesthetized rats.

2. Materials and methods

2.1. 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 of the International Association for the Study of Pain. Experiments were carried out on 112 male Sprague–Dawley rats weighing between 300 and 400 g. Animals were divided into the dose-dependent MO-treated group ($n = 35$), the lodocaine-treated group ($n = 21$) and the mGluR-treated group ($n = 56$). They were maintained in a temperature-controlled room ($23 \pm 1\,^{\circ}C$) with a 12/12 h light/dark cycle. In each experiment, the experimenter was blind to the treatment group.

2.2. General procedures

Behavioural assessment of craniofacial muscle pain was performed in lightly anesthetized rat model as previously described [40]. After initial anesthesia with sodium pentobarbital (40 mg/kg, i.p.), one femoral vein was cannulated and connected to an infusion pump (Harvard Apparatus, Pump 22) 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 for the duration of 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 [40]. 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 [40].

2.3. Evaluation of craniofacial muscle pain

The present study examined ipsilateral hindpaw shaking behaviour evoked by MO stimulation of 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 behaviour, 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 s. Intramuscular injection of MO produced ipsilateral hindpaw shaking behavioural response. The MO-induced hindpaw shaking behaviour was quantified by counting the total number of shaking behaviour for 4 min 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.

2.4. Evaluation of craniofacial muscle inflammation

We evaluated craniofacial muscle inflammation after intramuscular injection of 5, 10, 15, or 20% of MO. We examined the extent of plasma extravasation of Evans' blue dye as an index of inflammation after examination of MO-induced hindpaw shaking behaviour. MO-induced extravasated Evans' blue dye bound to plasma protein was measured as described previously [10,24,25]. Evans' blue dye (1%, 50 mg/kg) was administered into a femoral vein 30 min after MO injection. Ten minutes after injection of Evans' blue dye, each rat was perfused through the heart with normal saline. Masseter muscles were dissected, weighed, and stored at $-20\,^{\circ}C$ until analyzed. The tissues were incubated overnight in a 7:3 mixture of acetone and 0.5% sodium sulphate solution at room temperature with intermittent shaking. After incubation, samples were centrifuged at 300 rpm for 10 min and the supernatant was separated. The samples were analyzed by spectrophotometric measurement of absorbance at 620 nm for the amount of Evans' blue dye present. The recovery of the extravasated dye per gram weight of tissue (μg/g) was calculated by comparing the absorbency of the supernatant with a standard curve. The standard curve was generated from a series of the same extraction solution mixed with Evans' blue dye.

2.5. Effects of pretreatment with lidocaine on nociceptive behaviour and inflammation in the craniofacial muscle

The present study demonstrated that intramuscular injection of MO produced ipsilateral hindpaw shaking behavioral responses and increases in extravasated Evans' blue dye concentration. We investigated whether a local anesthetic inhibited MO-induced nociceptive behaviour and inflammation in the masseter muscle. Lidocaine (3 or 5%, 100 μl) was administered intramuscularly 5 min prior to the injection of 20% of MO into the masseter muscle. Saline was used as the injection of control for lidocaine.

2.6. Role of peripheral group I mGluR in nociceptive behaviour and inflammation in the craniofacial muscle

We investigated the effects of peripheral group I mGluR on hindpaw shaking behavioral responses and extravasated Evans' blue dye concentration produced by intramuscular injection of MO. MCPG (5 mM, 100 μl), a non-selective group I/II mGluR antagonist, LY367385 (2.5 or 5 mM, 100 μl), a selective group I mGluR1 antagonist, or MPEP (2.5 or 5 mM, 100 μl), a selective group I mGluR5 antagonist, were injected into the masseter muscle 30 min prior to 20% of MO injection. After intramuscular injection of MO, hindpaw shaking behavioral responses and extravasated Evans' blue dye concentration were measured. Saline was used as the injection of control for MCPG, LY367385, and MPEP.

2.7. Chemicals

(*S*)-α-Methyl-4-carboxyphenylglycine (MCPG), a non-selective group I/II mGluR antagonist, (*S*)-(+)-α-amino-4-carboxyl-2-methylbenzeneacetic acid (LY367385), a selective group I mGluR1 antagonist, and 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective group I mGluR5 antagonist, were obtained from Tocris. Evans' blue dye, lidocaine, and MO (ally isothiocyanate) were purchased from Sigma. All drugs were dissolved in normal sterile saline except MO. MO was diluted with mineral oil.

2.8. 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 ± S.E.M.

3. Results

Animals maintained on light anesthesia showed no significant spontaneous hindpaw shaking behavioral responses prior to MO injection. Intramuscular injection of MO (5, 10, 15 or 20%) produced an immediate and intense ipsilateral hindpaw shaking behavioral response which lasted for several minutes with peak number of shakes occurring within 1 min after intramuscular injection (Fig. 1). The hindpaw shaking behav-

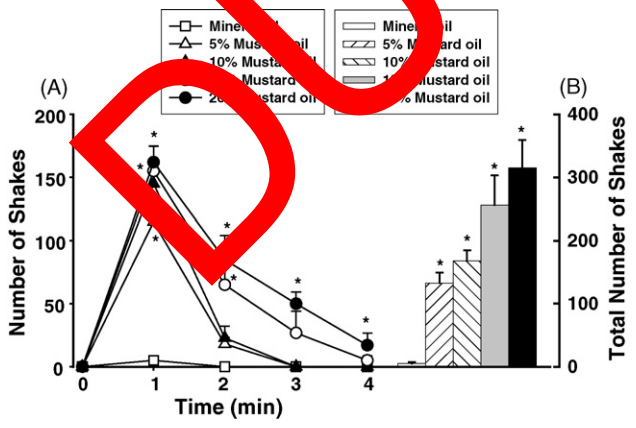

Fig. 1. Time course of MO-induced hindpaw shaking behavioral responses (A) and total number of shakes (B) in the masseter muscle. Animal received a 30 μl intra-muscular injection of 5, 10, 15 or 20% MO into the masseter muscle. The number of hindpaw shaking behaviour was measured for 4 min. There were seven animals in each group. $^{*}p < 0.05$, mineral oil vs. MO-induced hindpaw shaking behaviour.

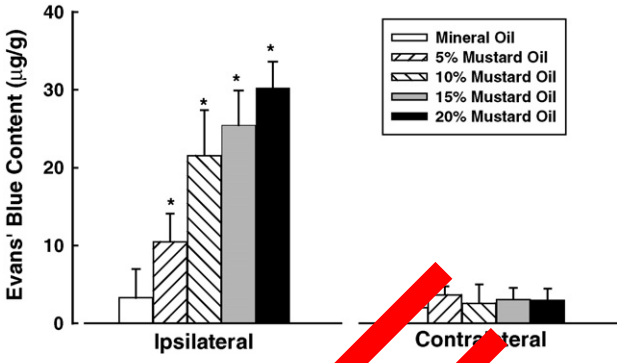

Fig. 2. MO-induced masseter muscle inflammation indicated by plasma extravasation. Evans' blue dye was extracted from inflamed masseter tissue and measured with a spectrophotometer at 620 nm. There were seven animals in each group. $^{*}p < 0.05$, mineral oil vs. MO-induced extravasated Evans' blue dye concentration.

ioral response appeared to be directed to the injected site as an attempt to rub or scratch the affected region. After injection of 5, 10, 15, or 20% MO into the masseter muscle, the total number of shaking behaviour was 133 ± 17, 168 ± 18, 256 ± 47, or 3[illegible] ± 44 number of scratches, respectively, in a dose-dependent manner and was significantly higher in the MO-treated group compared with the vehicle (mineral oil)-treated group ($p < 0.05$). 30 μl mineral oil did not evoke the hindpaw shaking behaviour in [illegible] of the animals tested.

The present study examined whether intramuscular injection of MO produced muscle inflammation. Changes in extravasated Evans' blue dye concentration in the masseter muscle ipsilateral to the injection site after intramuscular injection of MO are illustrated in Fig. 2. After intramuscular injection of mineral oil, the extravasated Evans' blue dye concentration was 4 ± 4 μg/g. However, the extravasated Evans' blue dye concentration was significantly greater in the MO-treated group compared with the vehicle (mineral oil)-treated group. Intramuscular injection of 5, 10, 15, or 20% MO increased the concentration of Evans' blue dye in the masseter muscle ipsilateral to the injection site to 11 ± 4, 22 ± 6, 26 ± 5, or 30 ± 4 μg/g, respectively, in a dose-dependent manner ($p < 0.05$). Intramuscular injection of MO did not affect the extravasated Evans' blue dye concentration in the masseter muscle contralateral to the injection site compared with the vehicle (mineral oil)-treated group.

We investigated whether intramuscular pretreatment with lidocaine, a local anesthetic, inhibited MO-induced nociceptive behaviour and inflammation in the masseter muscle. Effects of lidocaine injected intramuscularly on the total number of behavioral responses and the extravasated Evans' blue dye concentration produced by injection of MO are illustrated in Fig. 3. Intramuscular administration of vehicle (saline) did not affect MO-induced hindpaw shaking behavioral responses and extravasated Evans' blue dye concentration. Intramuscular pretreatment with 3 or 5% lidocaine significantly reduced MO-induced hindpaw shaking behaviour and increases in the extravasated Evans' blue dye concentration ($p < 0.05$) compared with vehicle-treated group. However, there were no differences between the 3 and 5% lidocaine-treated groups.

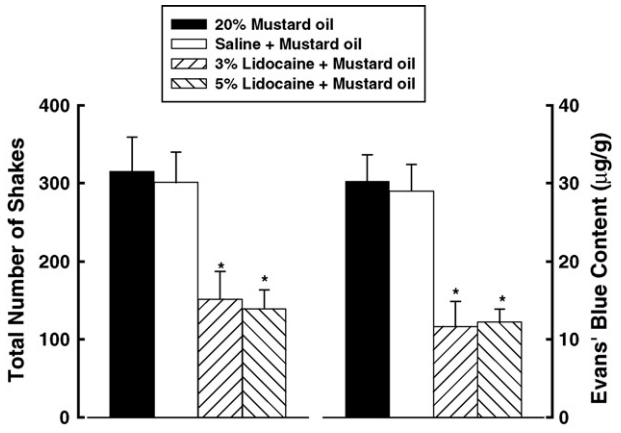

Fig. 3. Effects of intramuscular pretreatment with lidocaine on MO-induced hindpaw shaking behaviour and extravasated Evans' blue dye in the masseter muscle ipsilateral to injection site. Lidocaine was administered intramuscularly 5 min prior to the injection of 20% MO into the masseter muscle. Intramuscular pretreatment with 3 or 5% lidocaine significantly reduced MO-induced hindpaw shaking behaviour and concentration of Evans' blue dye ($p < 0.05$). There were seven animals in each group. $^{*}p < 0.05$, saline + MO- vs. lidocaine + MO-treated group.

We investigated the participation of peripheral group I mGluRs in MO-induced nociceptive behaviour and inflammation in the masseter muscle. Effects of MCPG, a non-selective group I/II mGluRs antagonist, on hindpaw shaking behavioral responses and extravasated Evans' blue dye concentration produced by MO injection are illustrated in Fig. 4. Intramuscular administration of vehicle (saline) did not affect MO-induced hindpaw shaking behavioral responses and increases in extravasated concentration of Evans' blue dye. Intramuscular pretreatment with MCPG, a non-selective group I/II mGluRs antagonist, significantly attenuated MO-induced hindpaw shaking behavior and increases in extravasated Evans' blue dye concentration compared with the vehicle-treated group ($p < 0.05$). However, intramuscular

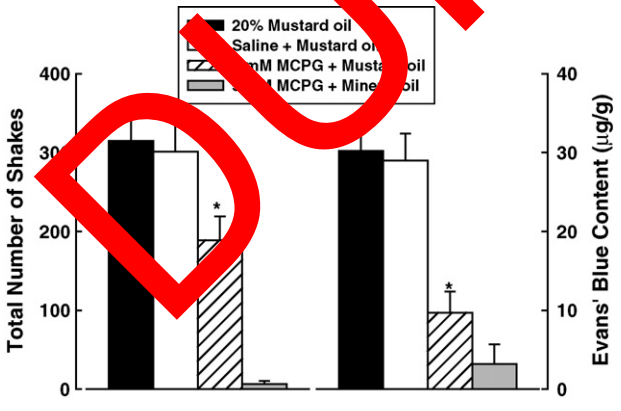

Fig. 4. Effects of intramuscular pretreatment with MCPG, non-selective group I/II mGluRs antagonist, on MO-induced hindpaw shaking behaviour and extravasated Evans' blue dye in the masseter muscle ipsilateral to injection site. MCPG was administered intramuscularly 30 min prior to the injection of 20% MO into the masseter muscle. Intramuscular pretreatment with 5 mM MCPG significantly reduced MO-induced hindpaw shaking behaviour and concentration of Evans' blue dye. There were seven animals in each group. $^{*}p < 0.05$, saline + MO- vs. MCPG + MO-treated group.

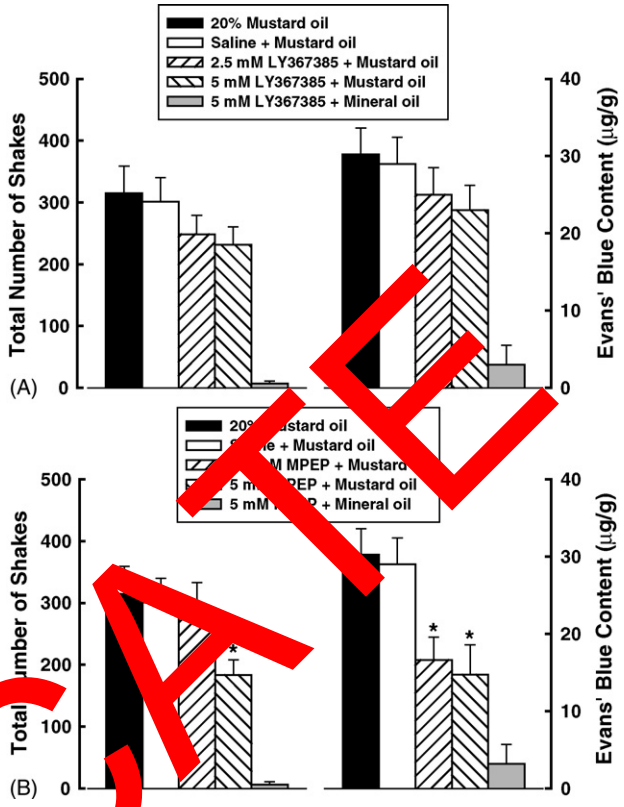

Fig. 5. The effects of intramuscular pretreatment with LY367385, a selective group I mGluR1 receptor antagonist (A), or MPEP, a selective group I mGluR5 receptor antagonist (B), on MO-induced hindpaw shaking behaviour and extravasated Evans' blue dye in the masseter muscle ipsilateral to injection site. MPEP was administered intramuscularly 30 min prior to the injection of 20% MO into the masseter muscle. Intramuscular pretreatment with 5 or 15 mM MPEP significantly reduced MO-induced hindpaw shaking behaviour and concentration of Evans' blue dye. There were seven animals in each group. $^{*}p < 0.05$, saline + MO- vs. MPEP + MO-treated group.

pretreatment with 5 mM MCPG alone produced no significant hindpaw shaking behaviour and extravasated Evans' blue dye concentration in the mineral oil-treated group. Effects of LY367385, a selective group I mGluR5 antagonist, or MPEP, a selective group I mGluR5 antagonist, on MO-induced hindpaw shaking behaviour and increases in extravasated Evans' blue dye concentration are illustrated in Fig. 5. Intramuscular pretreatment with 2.5 or 5 mM LY367385, a selective group I mGluR5 antagonist, did not affect MO-induced hindpaw shaking behaviour and increases in extravasated Evans' blue dye concentration compared with the vehicle-treated group. However, intramuscular pretreatment with 2.5 mM MPEP reduced increases in extravasated concentration of Evans' blue dye ($p < 0.05$), while it did not affect the total number of hindpaw shaking behavioral responses produced by MO injection. Intramuscular pretreatment with 5 mM MPEP produced a significant reduction of both the total number of shaking and extravasated concentration of Evans' blue dye compared with the vehicle-treated group ($p < 0.05$). However, intramuscular pretreatment with 5 mM MPEP alone produced no significant hindpaw shaking behaviour and extravasated Evans' blue dye concentration in the mineral oil-treated group.

4. Discussion

4.1. Intramuscular injection of MO produced nociceptive behaviour and inflammation in the craniofacial muscle

Recently, a new behavioral assessment method of the craniofacial muscle pain in the lightly anesthetized rat was introduced [40]. Ipsilateral hindpaw shaking behaviour was evoked by intramuscular injections with algesic agents in lightly anesthetized rats. The present study also demonstrated that intramuscular application of MO into the masseter muscle produced consistent and vigorous ipsilateral hindpaw shaking behavioral response in lightly anesthetized rats. Noxious stimulation of the orofacial region consistently produced immediate and intense episodes of asymmetrical grooming of the affected area with ipsilateral fore- or hindpaw in conscious rats [9,10,11,13,52]. Such asymmetric pain-related grooming activities are distinguished from prolonged and stereotyped symmetric grooming responses produced by non-noxious stimuli or mild irritation of body area [51]. Moreover, asymmetric grooming behaviour has been used as an assessment method of cutaneous facial pain [12,37]. In cutaneous facial pain, there is a positive relationship between the amplitude of the grooming responses and the concentrations of algesic chemicals. Finally, grooming or shaking behaviour was used to evaluate craniofacial muscle pain in lightly anesthetized rats [40,41]. The present study showed that intramuscular injection of MO produced a characteristic ipsilateral hindpaw shaking, mimicking the pain-induced grooming behaviour in intact rats, and that the hindpaw shaking behaviour appeared to be directed to the injected site. These results, taken together with previous data, suggest that evaluation of MO-induced ipsilateral hindpaw shaking behaviour is a valid measurement of the craniofacial muscle pain. In addition to ipsilateral hindpaw shaking behaviour, intramuscular injection of MO produced muscle inflammation. Plasma extravasated Evans blue dye concentration was measured as an index of inflammation. Intramuscular injection of 5, 10, 15, or 20% of MO significantly increased the extravasated Evans blue dye concentration in the masseter muscle compared with the vehicle-treated group. These results are consistent with previous data. Topical application of MO to the mouse ear produced acute inflammatory responses such as plasma extravasation and edema formation [27]. Previous data, taken together with the present data, indicate that intramuscular injection of MO into the masseter muscle produced inflammation accompanied with nociceptive behaviour.

4.2. Effects of lidocaine on MO-induced nociceptive behaviour and inflammation in the craniofacial muscle

It is well known that topical application of MO to the receptive fields of skin localized Evans' blue extravasation and the topical application MO excites small diameter afferents [38]. The present data demonstrated that intramuscular injection of MO produced both ipsilateral hindpaw shaking behaviour as pain responses and increases in Evans' blue dye concentration as a muscle inflammation. These results, taken together with previous data, indicate that peripheral injection of MO produce ipsilateral hindpaw shaking behaviour and increases in the concentration of Evans' blue dye through small fiber-mediated pain behavioral responses and neurogenic inflammation, respectively. Neurogenic inflammatory responses comprising vasodilatation, plasma extravasation, and mast cell activation are induced by electrical and chemical stimulation of sensory neurons [33]. These results are consistent with other previous studies, which demonstrated that neurogenic inflammation was not observed in a denervated component of the rat hind paw skin [4]. In the present study, 3 or 5% lidocaine significantly reduced MO-induced hindpaw shaking behaviour and increases in concentration of Evans' blue dye. Although MO-induced hindpaw shaking behaviour and increases in concentration of Evans' blue dye in the masseter muscle were reduced by pretreatment with local anesthetic, they were still high level compared with the vehicle-treated group. The present results are inconsistent with previous data. Pretreatment with local anesthetics reduced intra-articular injection of MO-induced jaw and neck muscle activity to the baseline level in human [58] and reduced neurogenic plasma extravasation to the baseline level in the skin of rats [4]. On the contrary, local anesthetics did not block MO-induced inflammation in temporomandibular joint [55]. Moreover, intramuscular pretreatment with lodocaine did not recover intramuscular injection of MO-induced hindpaw shaking behaviour to baseline level [40]. Finally, pretreatment with substance P antagonist into the masseter muscle did not block the edema formation produced by intramuscular injection of MO [42]. These results, taken together with the present data, indicate that although local anesthetics reduced MO-induced nociceptive behaviour and increases in concentration of Evans' blue dye in the masseter muscle, it did not recover to baseline level. These results imply that MO, which is known to cause a pure neurogenic inflammation in the cutaneous tissue, may induce pain and inflammation by a non-neurogenic component.

4.3. Effects of peripheral group I mGluRs antagonist on MO-induced nociceptive behaviour and inflammation in the craniofacial muscle

Recently, behavioral [20] and electrophysiological [32,56] evidence has indicated the involvement of central metabotropic glutamate group I (mGluR1 and mGluR5) receptors in nociception. In addition to its central action, group I mGluRs also participated in the pain processing of peripheral sites. The injection of a selective mGluR5 agonist into the naive rat hindpaw produced mechanical hyperalgesia and inflammatory hyperalgesia, and mGluR-induced responses were inhibited by pretreatment with MPEP, group I mGluR5 antagonist [53,54]. Intraplantar injection of MPEP, group I mGluR5 antagonist, produced analgesic effects in the skin-incision-induced post-operative pain model in rats [60]. However, the involvement of peripheral group I mGluRs in craniofacial muscle pain has not been defined yet. The present study first demonstrated the involvement of peripheral group I mGluRs in MO-induced nociceptive behaviour and inflammation in the masseter muscles. Intramuscular pretreatment with MCPG, a non-selective group I/II mGluRs antago-

nist, or MPEP, a selective group I mGluR5 antagonist, significantly reduced MO-induced hindpaw shaking behaviour, while LY367385, a selective group I mGluR1 antagonist, did not affect MO-induced responses. However, the same dose of mGluR antagonist injected into the biceps muscle did not produce significant effects on MO-induced hindpaw shaking behaviour and pretreatment with mGluRs alone also produced no significant hindpaw shaking behaviour and extravasated Evans' blue dye concentration in the mineral oil-treated group. These results indicate a major contribution of group I mGluR5 in mediating craniofacial muscle pain. The present data are consistent with previous data that intra-plantar injection of MPEP, a selective group I mGluR5 antagonist, attenuated mechanical hyperalgesia and spontaneous nociceptive behaviour following inflammation [5] and produced analgesic effects in the skin-incision-induced postoperative pain model in rats [60]. The blockade of peripheral mGluR5 of sensory afferents before the noxious insult prevented the development of the process leading to inflammatory pain [5], suggesting that peripheral mGluR5 mediates a pivotal role in the nerve sensitization linked with inflammatory conditions. However, the exact underlying mechanisms of involvement of peripheral mGluRs in MO-induced nociceptive behaviour in the craniofacial muscle is still not clear. One possible mechanism is that peripheral mGluR antagonist reduces continuous autogenic activation of muscle nociceptors by glutamate, and that release of glutamate from nerve endings is triggered by MO stimulation. In fact, there is evidence that glutamate, released in the peripheral hindpaw after inflammation, is a key mediator of inflammation-evoked hyperalgesia [16]. Experimental induction of joint inflammation in rats has been shown to induce an intense and prolonged release of glutamate from sensory neurons through activation of dorsal root reflexes [31,39]. Intramuscular injection of glutamate into the masseter muscle excited and sensitized masseter muscle afferent fibers through activation of peripheral excitatory amino acid receptors in anesthetized rats [6] and produced increases in jaw and neck muscle activity and mechanical allodynia in human subjects [4,9,50]. Additionally, pretreatment with MK-801, an NMDA receptor antagonist, abolished the MO-induced nocifensive behaviour [41] and reduced MO-induced increases in the electromyography activity of the masseter and the digastric muscles and Evans' blue plasma extravasation in rats [57]. Taken the present data, peripheral group I mGluR5 plays major role in mediating nociceptive behaviour in the craniofacial muscle, suggesting that multiple receptor systems are activated in parallel following muscle tissue injury. Therefore, assessment of the relative contribution from each receptor type will prove beneficial for treatment of craniofacial muscle pain.

The present study demonstrated that intramuscular pretreatment with MPEP, an selective mGluR5 antagonist, significantly decreased extravasated Evans' blue dye concentration induced by MO injection, while LY367385, an selective mGluR1 antagonist, did not affect MO-induced extravasated Evans' blue dye concentration. However, the same dose of mGluR antagonist injected into the biceps produced no significant effects on MO-induced extravasated Evans' blue dye concentration. These results indicate that peripheral group I mGluR5 play important roles in mediating craniofacial muscle inflammation. These results are supported by previous data, which has demonstrated that glutamate, an excitatory amino acid, injected into the rat masseter muscle produces a significantly greater edema volume and extracellular water content compared to those produced by isotonic control injection [6]. On the contrary, direct activation of peripheral excitatory amino acid receptors is not sufficient to induce tissue inflammation. Direct injection of glutamate into the TMJ or rat hindpaw does not cause plasma extravasation or edema formation [15,19], although an increased level of glutamate and other amino acids such as arginine and citrulline has been proposed as providing potent proinflammatory actions [31]. However, the present data demonstrate that intramuscular injection of MO produces inflammation accompanied with nociceptive behaviour in the craniofacial muscle. These result indicate that peripheral group I mGluR5 also play important roles in mediating MO-induced craniofacial muscle inflammation.

Since the peripheral group I mGluR5 effectively inhibits the MO-induced nociceptive behaviour as well as inflammation in the masseter muscle, it may prove useful to target these peripheral receptors for analgesic and anti-inflammatory therapies. Further studies involving selective activation or blockade of other metabotropic glutamate receptor subtypes will reveal the relative contribution of different receptor systems or interaction between them in mediation nociceptive behaviour and inflammation in the craniofacial muscle.

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