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Blockade of central cyclooxygenase (COX) pathways enhances the cannabinoid-induced antinociceptive effects on inflammatory temporomandibular joint (TMJ) nociception

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Abstract

The present study is the first to investigate the participation of central cyclooxygenase (COX) pathways in modulating the antinociceptive effects of intracisternally administered cannabinoid on nociception induced by inflammation of the temporomandibular joint (TMJ) in freely moving rats. Following intra-articular injection of 5% formalin in the TMJ, nociceptive scratching behavior was recorded for nine successive 5-min intervals in Sprague–Dawley rats. Intracisternal injection of 30 μg of WIN 55,212-2, a synthetic non-subtype-selective CB1/2 agonist, administered 20 min prior to formalin injection significantly reduced the number of scratches and duration of scratching induced by formalin compared with the vehicle-treated group. Antinociceptive effect of WIN 55,212-2 was blocked by intracisternal injection of 10 μg of AM251, a CB1 receptor-selective antagonist, but not by AM630, a CB2 receptor-selective antagonist. A 10 μg dose of WIN 55,212-2 that was ineffective in producing antinociception became effective following intracisternal administration of NS-398, a selective COX-2 inhibitor; indomethacin, a non-selective COX 1/2 inhibitor; acetaminophen, a putative COX-3 inhibitor, but not following pretreatment with the selective COX-1 inhibitor, SC-560. The ED_{50} value of WIN 55,212-2 in the NS-398-treated group was significantly lower than that in the vehicle-treated group. Importantly, administration of low doses of COX inhibitors alone did not attenuate nociception. These results indicate that inhibition of central COX pathways, presumably via COX-2 inhibition, reduces inflammatory pain by enhancing the cannabinoid-induced antinociceptive effect. Based on our observations, combined administration of cannabinoids with COX inhibitors may hold a therapeutic promise in the treatment of inflammatory TMJ pain.

Keywords: Antinociception; Cannabinoid; COX inhibitor; Formalin; Hyperalgesia; TMJ

1. Introduction

Following the discovery of an endogenous ligand for the cannabinoid receptor, an intense investigation led to the identification and cloning of two distinct cannabinoid receptors; CB1 that is predominantly found in the central nervous system (Matsuda et al., 1990), and CB2 that is found in cells of the immune system (Munro

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et al., 1993). Cannabinoid receptors are localized in anatomical regions involved in nociceptive transmission and modulation, such as the dorsal horn of the spinal cord and the periaqueductal gray (Herkenham et al., 1991; Tsou et al., 1998). Behavioral studies in animals indicate that cannabinoid receptors are involved in the suppression of pain transmission (Sofia et al., 1973; Moss and Johnson, 1980; Herzberg et al., 1997). Intrathecal administration of cannabinoid receptor agonists has been reported to produce anti-hyperalgesic and anti-allodynic effects in neuropathic and inflammatory pain, respectively (Martin et al., 1999; Fox et al., 2001). Electrophysiological studies also have demonstrated that cannabinoid receptor agonists inhibit the activity of nociceptive neurons in the dorsal horn of the lumbar spinal cord (Drew et al., 2000; Chapman, 2001; Kelly and Chapman, 2001). Intraperitoneal administration of Δ^9-tetrahydrocannabinol (THC) and anandamide, a natural cannabinoid and an endocannabinoid, respectively, produces antinociception in the paw pressure test in arthritic rats (Smith et al., 1998). Recently, an antinociceptive effect of cannabinoids also has been reported in the orofacial region. Local application of WIN 55,212-2, a synthetic non-subtype-selective cannabinoid CB1 and CB2 agonist, significantly reduced the activity of wide dynamic range (WDR) neurons in the medullary dorsal horn (trigeminal subnucleus caudalis) in rats (Papanastassiou et al., 2004). These observations indicate the potential usefulness of cannabinoid receptor agonists in the treatment of orofacial pain conditions.

Cyclooxygenase (COX) inhibitors are known to produce analgesic effects through the suppression of synthesis of prostaglandins in the periphery (Vinegar et al., 1976). However, recent studies also indicate the participation of central COX pathways in the processing of nociceptive information in the spinal cord. For example, intrathecal administration of COX-2 inhibitors was reported to decrease inflammation-induced PGE_2 levels in the spinal cord of rats (Samad et al., 2001). More recently, central COX pathways were shown to modulate actions of endocannabinoids in the brain (Kim and Alger, 2004). In hippocampal pyramidal cells, inhibition of COX pathways enhanced the inhibitory effect of endocannabinoids on GABAergic transmission. The present study addressed the hypothesis that central COX pathways might also modulate the antinociceptive effects of cannabinoids on inflammation-induced temporomandibular joint (TMJ) nociception. The antinociceptive effect of WIN 55,212-2, a synthetic non-subtype-selective CB1/2 receptor agonist, was tested on the formalin-induced TMJ nociception with or without prior intracisternal administration of subtype selective and non-selective COX inhibitors. Parts of this work have been published previously in abstract form (Choi et al., 2005a).

2. Materials and methods

2.1. Animals

Experiments were carried out on 280 male Sprague–Dawley rats weighing 220–280 g. The animals were maintained in a temperature-controlled room (23 ± 1 °C) with a 12/12 h light–dark cycle. All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of the School of Dentistry, Kyungpook National University and were carried out in accordance with the ethical guidelines for the investigation of experimental pain in conscious animals proposed by the International Association for the Study of Pain. All behavioral responses were measured by an experimenter who was blind to the treatment group.

2.2. Experimental protocol

The aim of the first set of experiments was to examine antinociceptive effect of intracisternally administered WIN 55,212-2 on formalin-induced TMJ nociception. Rats were randomly assigned to one of five groups in this and subsequent set of experiments and there were eight animals in each group. The behavior of rats, treated with saline or formalin, was monitored and 10 or 30 μg of WIN 55,212-2-treated group was compared with the vehicle-treated group. A second set of experiments was performed in order to investigate which cannabinoid receptor is involved in WIN 55,212-2-induced antinociception. The vehicle ($n = 8$) was administered as a control. The behavior of rats, treated with 10 μg of AM251 ($n = 8$), and 10 μg ($n = 8$) or 100 μg ($n = 8$) of AM630, was monitored in the 30 μg of WIN 55,212-2-treated group. The behavior of rats, treated with 10 μg of AM251 ($n = 8$) or 100 μg ($n = 8$) of AM630, was monitored in the vehicle-treated groups. A third set of experiments was performed to investigate the participation of central opioid pathways in the cannabinoid-induced antinociception. After intracisternal administration of the vehicle ($n = 8$) or naloxone ($n = 8$), changes in the number of scratches and duration of scratching were examined in the 30 μg of WIN 55,212-2-treated groups. A fourth set of experiments was performed to investigate effects of co-administration of low doses of COX inhibitors and WIN 55,212-2. The behavior of rats, treated with the vehicle, SC-560, NS-398, indomethacin, or acetaminophen, was examined in the 10 μg of WIN 55,212-2-treated groups. We also investigated behavior of rats, treated with vehicle, SC-560, NS-398, indomethacin, or acetaminophen, in the formalin-induced TMJ nociception without pretreatment with 10 μg of WIN 55,212-2. A fifth set of experiments was performed to investigate effects of intracisternal pretreatment with 30 μg of NS-398 on dose-dependent antinociceptive effects of WIN 55,212-2. Antinociceptive effects of intracisternal administration of 1, 3, 10, 30 or 80 μg of WIN 55,212-2 were examined in the vehicle or NS-398-treated groups, respectively. We also examined Evan's blue dye concentration after injection of formalin into the masseter mussel ($n = 8$) or contralateral TMJ ($n = 8$).

2.3. Intra-articular injection of formalin into the TMJ

Each animal was first placed in a Plexiglas box for a 30 min period so as to minimize stress (Roveroni et al., 2001; Ahn et al., 2005). Rats were not allowed access to food or water

during the test. Animals were placed in a box and anesthetized with 5% halothane for TMJ injection. Animals were removed from the box as soon as they were anesthetized. It takes 20–30 s for TMJ injection, and animals usually recovered from anesthesia within 2–3 min after the TMJ injection. A formalin test was applied to the TMJ region with modifications, as described previously (Roveroni et al., 2001; Ahn et al., 2005; Choi et al., 2005b). The injections into the TMJ region were performed via a 30-gauge needle introduced into the TMJ capsule of the left TMJ, as previously described by us and others (Roveroni et al., 2001; Ahn et al., 2005; Choi et al., 2005b). A cannula consisting of a polyethylene tube was connected to the needle and also to a Hamilton syringe (50 μL) previously filled with formalin solution. The volume of the TMJ injections was 50 μL. For each animal, the number of noxious behavioral responses and the time spent grooming, rubbing, and/or scratching the TMJ region were recorded for nine successive 5-min intervals (Roveroni et al., 2001; Ahn et al., 2005). To minimize the possibility that the behavior produced by formalin might have resulted from its effect on regions outside the TMJ, off-site injections were performed as described previously (Roveroni et al., 2001; Ahn et al., 2005; Choi et al., 2005b). The same volume of formalin was injected into the right masseter muscle. Saline was injected into the TMJ region as a control. Behavioral responses in the formalin-induced pain model generally display two distinct phases (Clavelou et al., 1989, 1995; Choi et al., 2003a,b,c). However, behavioral responses induced by formalin injection in the TMJ did not display the two distinct phases because the early phase was masked by anesthesia (Ahn et al., 2005). The actual observations started 11 min after the formalin injection. We analyzed the total number of scratches and duration of scratching in the second phase (11–45 min, 2nd phase) as indices of TMJ nociception after formalin injection (Roveroni et al., 2001; Ahn et al., 2005; Choi et al., 2005b).

2.4. Effect of WIN 55,212-2 injected intracisternally on formalin-induced TMJ pain

2.4.1. Placement of the intracisternal catheter

Animals were anesthetized with pentobarbital sodium (40 mg/kg, ip). The anesthetized rats were individually mounted on a stereotaxic frame and a polyethylene tube (PE10) was implanted for the intracisternal injection, as described previously (Yaksh and Rudy, 1976; Ahn et al., 1998, 2005; Wang et al., 2002). The polyethylene tube was subcutaneously led to the top of the skull and secured in place by a stainless steel screw and dental acrylic resin.

2.4.2. Intracisternal administration of WIN 55,212-2

After a 72-h recovery period from surgery, WIN 55,212-2 (10 or 30 μg/10 μL) was administered intracisternally through an implanted PE tube 20 min prior to injection of formalin in freely moving rats. After 50 μL of 5% formalin was injected into the TMJ region, rats were returned to the test chamber for a 45 min observation period. We examined changes in the number of scratching responses and the time spent grooming, rubbing, and/or scratching the TMJ region which were produced by the injection of formalin into the TMJ region. In the control group, 10 μL of the vehicle of WIN 55,212-2 (80% of DMSO/saline) was injected intracisternally. For confirmation of the placement of the cannula and the extent of the spread of drugs, pontamine sky blue dye was injected at the end of the tests. Intrathecal catheterization may produce motor dysfunction. Therefore, we examined whether intracisternal catheter produced motor dysfunction or not. After implantation of intracisternal catheter, motor functions of animals were evaluated by observation of normal movements and rotarod test. Only animals that displayed normal motor functions were evaluated. We excluded animals that showed malposition of catheter after intrathecal catheterization and 2.5% were excluded due to apparent motor dysfunctions, outlined above.

2.5. Effects of cannabinoid receptor antagonists injected intracisternally on cannabinoid-induced antinociception

In order to investigate which receptor subtype mediates cannabinoid-induced antinociception, AM251 (10 μg/10 μL), a CB1 receptor antagonist, or AM630 (10 or 100 μg/10 μL), a CB2 receptor antagonist, was administered intracisternally 10 min prior to the application of WIN 55,212-2. The present study also investigated the involvement of opioid pathways in cannabinoid-induced antinociception. After the intracisternal injection of naloxone (30 μg/10 μL), an opioid receptor antagonist, 10 min prior to administration of cannabinoid, formalin-induced nociceptive behavior was examined. In the control group, 10 μL of the vehicle of AM251 (80% DMSO/saline), AM630 (80% DMSO/saline), or naloxone (saline) was injected intracisternally.

2.6. Role of central COX pathways in cannabinoid-induced antinociception

In order to investigate the role of central COX pathways in cannabinoid-induced antinociception, various COX inhibitors were administered intracisternally. SC-560 (30 μg/10 μL), a selective COX-1 inhibitor, NS-398 (30 μg/10 μL), a selective COX-2 inhibitor, indomethacin (30 μg/10 μL), a non-selective COX1/2 inhibitor, or acetaminophen (ATAP, 30 μg/10 μL), a putative COX-3 inhibitor, was administered through the cisternal catheter 10 min prior to the intracisternal administration of 10 μg of WIN 55,212-2. In the control group, 10 μL of the vehicle for COX inhibitors (80% of DMSO/saline) was injected intracisternally. We investigated the dose-dependent antinociceptive effects of WIN 55,212-2 (1, 3, 10, 30 or 80 μg/10 μL), which was injected intracisternally, after intracisternal pretreatment with 30 μg of NS-398, a selective COX-2 inhibitor. Data analysis involved sigmoidal non-linear regression curve fitting for the dose–response data and the estimation of ED_{50} of WIN 55,212-2.

2.7. Verification of inflammation

The formalin-induced plasma extravasation of Evans' blue dye which bound to plasma protein was measured, as described previously (Harada et al., 1971; Haas et al., 1992; Cairns et al., 1998; Ahn et al., 2005; Choi et al., 2005b). This procedure confirmed that the plasma extravasation, which was induced by the TMJ injection, was indeed restricted to the TMJ region. At the conclusion of each experiment,

the animals were anesthetized with pentobarbital sodium (40 mg/kg, ip). Evans' blue dye (0.1%, 5 mg/kg) was injected into the right femoral vein. Ten minutes after the injection of the dye, each rat was perfused with heparinized normal saline. Joint tissues were dissected from the right side, weighed and stored at −20 °C until analyzed. The tissues were incubated overnight in a 7:3 mixture of acetone and 5% sodium sulphate solution at room temperature with intermittent shaking. After incubation, the samples were centrifuged at 300 rpm for 10 min and the supernatant was separated. The samples were analyzed for the amount of dye present by spectrophotometrically measuring absorbance at 620 nm. The recovery of the extravasated dye per gram weight of tissue (μg/g) was calculated by comparing the absorbance of the supernatant with a standard curve. The standard curve was generated from a series of the same extraction solution mixed with known amounts of Evans' blue dye.

2.8. Rotarod test

Changes in motor performance, after the intracisternal administration of 10 or 30 μg of WIN 55,212-2, were measured using an accelerating rotarod (Ugo Basil, Comerio), as described previously (Blackburn-Munro et al., 2004). The rotarod speed was increased from 8 to 16 rpm over a 180 s period, with the maximum time spent on the rod set at 180 s. Rats received two or three training trials on two separate days prior to testing for acclimatization. On the experimental day, the resting response was examined. After the intracisternal administration of WIN 55,212-2 or the vehicle (80% DMSO), the time course of the motor performance was examined.

2.9. Chemicals

WIN 55,212-2, AM251, and AM630 were dissolved in 80% DMSO and 20% sterile saline. SC-560, NS-398, indomethacin, and acetaminophen were dissolved in 80% DMSO and 20% sterile saline. WIN 55,212-2, AM251, and AM651 were obtained from Tocris-Cookson and SC-560, NS-398, indomethacin, and acetaminophen were obtained from Sigma. All intracisternal injections were made through an implanted PE tube. The PE tube had a dead space volume of 7 and 8 μL of saline was injected to flush the cannula following each microinjection.

2.10. Statistical analysis

Statistical analysis of the behavioral data was carried out with a one-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Comparisons between the two means were performed by a Student's *T*-test. Sigmoidal non-linear regression curve fitting for dose–response data and the estimation of ED_{50} of WIN 55,212-2 were fitted using a least-squares fit to the following equation: $I = I_{max} - I_{max} \times [C^n/(C^n + IC_{50}^n)]$, where I is the inhibitory effect of WIN 55,212-2, C is the concentration of WIN 55,212-2, IC_{50} is the concentration of WIN 55,212-2 that produced 50% of the maximum possible effect and n is the Hill coefficient. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance. All data are presented as means ± SEM.

3. Results

3.1. Extent of spread of inflammation induced by formalin injection in the TMJ

Evans' blue dye (0.1%, 5 mg/kg) was injected systemically to determine the extent of spread of inflammation induced by injection of formalin in the TMJ. Fig. 1 illustrates the average concentration level of Evans' blue dye in tissues obtained from TMJ, treated by formalin or saline. The level of dye was significantly higher in the formalin-treated group ($p < 0.05$) as compared to the saline-treated group (Fig. 1). However, the amount of dye on the contralateral TMJ side did not differ from that of the saline-treated group. Moreover, an intramuscular injection of formalin did not increase the amount of Evans' blue dye in TMJ. These findings affirm the validity of our TMJ model of inflammatory pain for the assessment of nociceptive and antinociceptive responses.

3.2. Nociceptive behavior induced by formalin injection in the TMJ is inhibited by a cannabinoid agonist through activation of CB1

In order to determine whether cannabinoids modulate inflammation-induced TMJ nociception, effects of an intracisternally administered cannabinoid agonist were tested on the nociceptive behavior induced by formalin injection in the TMJ. Fig. 2 illustrates the antinociceptive effect of intracisternally administered WIN 55,212-2, a synthetic non-subtype-selective CB1/2

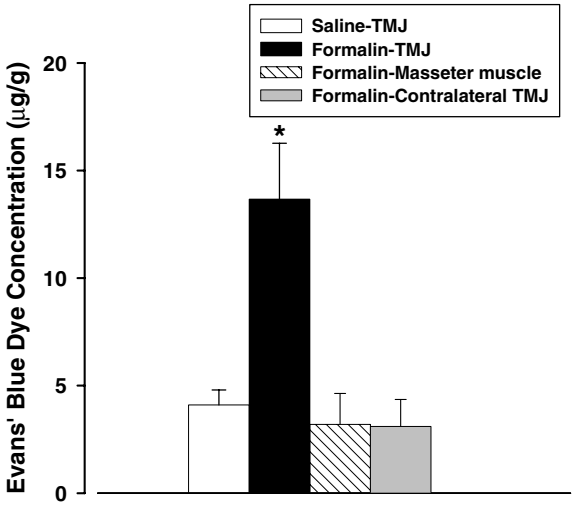

Fig. 1. Measurement of the location of the inflammatory response based on assessment of plasma protein extravasation using Evans' blue dye. Saline-TMJ, saline injected into TMJ; Formalin-TMJ, formalin injected into TMJ; Formalin-masseter muscle, formalin injected into masseter muscle; Formalin-contralateral TMJ, dye concentration of contralateral TMJ. There were eight animals in each group. *$p < 0.05$, Formalin-TMJ- vs. Saline-TMJ-treated group.

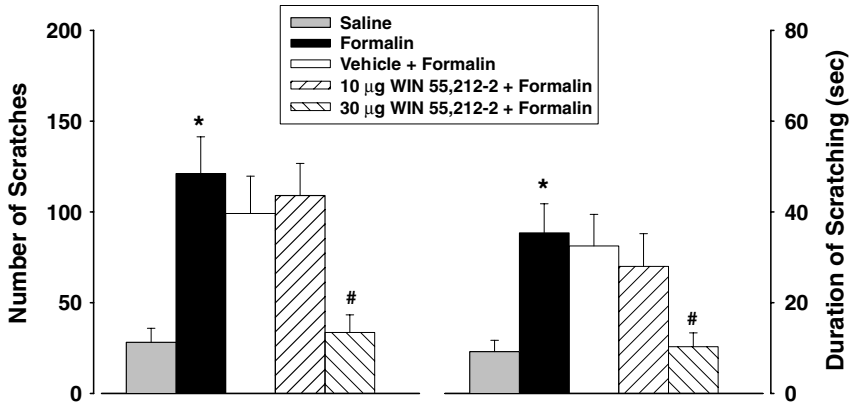

Fig. 2. Cannabinoid agonist (WIN 55,212-2) reduces the number of scratches and the duration of scratching produced by an intra-articular injection of formalin into TMJ. Intracisternal injection of 10 μg, or 30 μg of WIN 55,212-2, a non-subtype-selective CB1/2 receptor agonist, occurred 20 min prior to the formalin injection. Microinjection of 50 μl of 5% formalin into the TMJ significantly produced noxious scratching behavioral responses. There were eight animals in each group. *$p < 0.05$, Saline vs. Formalin-treated group. $^{\#}p < 0.05$, WIN 55,212-2 + Formalin- vs. Vehicle + Formalin-treated group.

receptor agonist, on the number of scratches and the duration of scratching produced by formalin injection into the TMJ region. Intra-articular injection of 50 μL of 5% formalin produced noxious scratching behavior, which lasted for 40 min ($p < 0.05$). Neither the vehicle nor a 10 μg dose of WIN 55,212-2 altered the formalin-induced scratching behavior. However, intracisternal injection of 30 μg of WIN 55,212-2 attenuated the number of scratches and duration of scratching by 64% and 69% (42 ± 15 in the number of scratches and 13 ± 4 s, $p < 0.05$), respectively, as compared with the vehicle-treated group.

In the present study, motor functions were evaluated with a rotarod test after the intracisternal administration of vehicle (80% DMSO and 20% sterile saline) or WIN 55,212-2. Neither intracisternal administration of vehicle nor 10 or 30 μg of WIN 55,212-2 affected motor functions compared with that of the naïve rats.

The effects of the intracisternal administration of cannabinoid receptor antagonists on WIN 55,212-2-induced antinociception are illustrated in Fig. 3. Intracisternal administration of 10 μg of AM251 or 100 μg of AM630, by itself, did not affect the scratching behavior which was produced by intra-articular injection of formalin. The vehicle did not affect the formalin-induced scratching behavior in the TMJ region. However, intracisternal injection of 10 μg of AM251 blocked WIN 55,212-2-induced antinociception ($p < 0.05$), while intracisternal pretreatment with 10 or 100 μg of AM630 did not alter WIN 55,212-2-induced antinociception.

The present study also investigated the participation of central opioid pathways in the cannabinoid-induced antinociception. Intracisternal pretreatment with naloxone (30 μg), an opioid receptor antagonist, did not block the suppression of the number of scratches and the duration of scratching produced by the intracisternal injection of WIN 55,212-2 (Fig. 4). Intracisternal administration of naloxone alone did not alter the formalin-induced nociceptive behavior.

3.3. Central Blockade of COX-2 and COX-3 pathways enhances the antinociceptive effects of a cannabinoid agonist

In order to understand the role of central COX pathways in modulating cannabinoid-induced antinociception, the effect of an ineffective dose (10 μg) of WIN 55,212-2 was tested in the presence of central blockade of COX pathways (Fig. 5). Intracisternal administration of 10 μg of WIN 55,212-2 produced a significant reduction in the number and duration of scratching behavior induced by formalin following intracisternal pretreatment with 30 μg of indomethacin, a non-selective COX1/2 inhibitor or NS-398, a selective COX-2 inhibitor or acetaminophen, a putative COX-3 inhibitor, but not SC-560, a selective COX-1 inhibitor (Fig. 5). Pretreatment with the vehicle for COX inhibitors did not alter the lack of effect of 10 μg of WIN 55,212-2 on formalin-induced scratching behavior in the TMJ regions. It is also important to note that administration of COX inhibitors alone did not affect the formalin-induced nociceptive scratching behavior as compared with the vehicle-treated group (Fig. 6). Thus, effects of anti-inflammatory agents are principally due to enhancement of cannabinoid-induced antinociception.

Our data showed that intracisternally administered indomethacin or NS-398 but not SC-560 produced antinociception in the 10 μg of WIN 55,212-2-treated group. These results suggest that central COX 2 pathway plays an important role in the antinociception of central cannabinoid. We further investigated the effects of blockade of central COX2 pathway on dose–response curves for the antinociceptive effects of WIN 55,212. A comparison of the dose–response curves for the antinociceptive

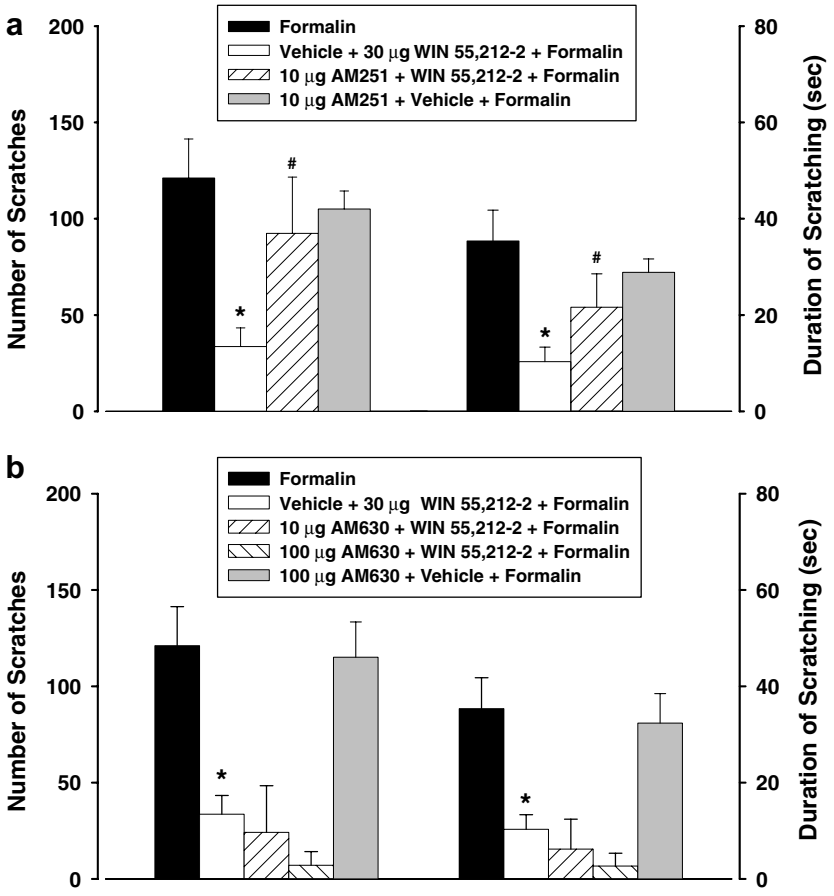

Fig. 3. Intracisternal injection of a CB1 receptor antagonist (AM251, 10 μg), but not a CB2 receptor antagonist (AM630, 10 μg or 100 μg), blocked antinociception mediated by a 30 μg dose of WIN 55,212-2. (a) Pretreatment with AM251 reduced antinociception produced by the intracisternal administration of 30 μg of WIN 55,212-2. (b) Pretreatment with AM630 did not affect the antinociception produced by 30 μg of WIN 55,212-2. There were eight animals in each group. $^{*}p < 0.05$, Formalin- vs. Vehicle + WIN 55,212-2 + Formalin-treated group. $^{\#}p < 0.05$, Vehicle + WIN 55,212-2 + Formalin vs. AM251 or AM630 + WIN 55,212-2 + Formalin-treated group.

effects of WIN 55,212, between the NS-398- and the vehicle-treated group, is illustrated in Fig. 7. Pretreatment with NS-398, a selective COX-2 inhibitor, significantly enhanced the antinociceptive effects of WIN 55,212-2, compared with that of the vehicle-treated group

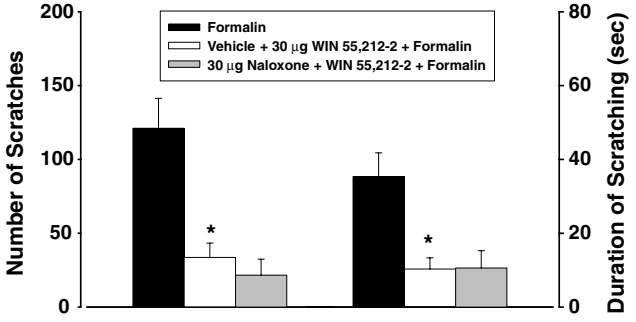

Fig. 4. Intracisternal injection of naloxone (30 μg), an opioid receptor antagonist, does not affect antinociceptive behavior in rats that were administered WIN 55,212-2 intracisternally. There were seven animals in each group. $^{*}p < 0.05$, Formalin- vs. Vehicle + WIN 55,212-2 + Formalin-treated group.

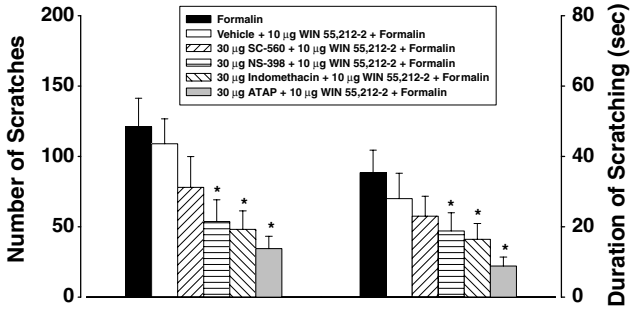

Fig. 5. Intracisternal pretreatment with COX inhibitors enhances sensitivity to low dose (10 μg) of WIN 55,212-2 on inflammation-induced scratching behavior. Intracisternal administration of 10 μg of WIN 55,212-2 alone did not produce antinociception. Pretreatment with a selective COX-2 inhibitor (NS-398, 30 μg), a non-isoform-selective COX inhibitor (indomethacin, 30 μg), or a tentative COX-3 inhibitor (acetaminophen, ATAP, 30 μg), produced antinociception in the 10 μg of WIN 55,212-2-treated group, as compared with the vehicle-treated group. There were eight animals in each group. $^{*}p < 0.05$, Vehicle + WIN 55,212-2 + Formalin vs. NS-398, indomethacin, or ATAP + WIN 55,212-2 + Formalin-treated group.

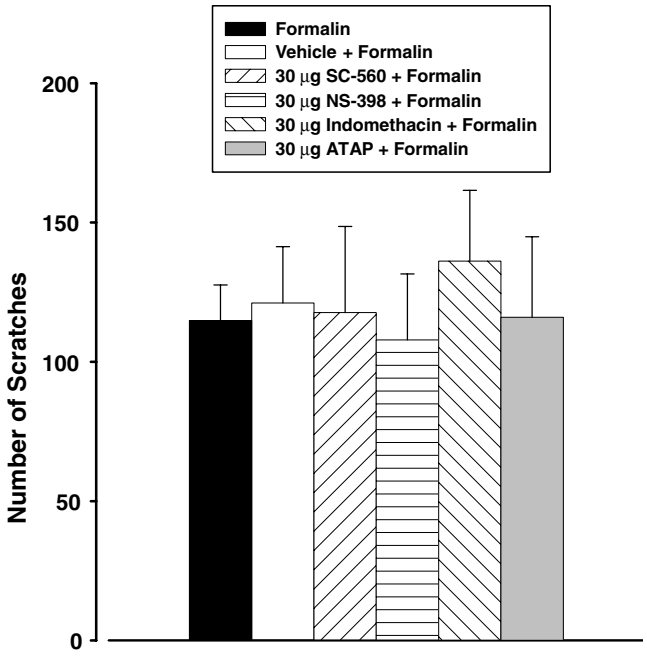

Fig. 6. Intracisternal administration of COX inhibitors alone does not reduce inflammation-induced TMJ nociception. Behavioral responses produced by an intra-articular injection of formalin into the TMJ after intracisternal injection of the vehicle (70% DMSO and 30% saline), SC-560, a selective COX-1 inhibitor; NS-398, a selective COX-2 inhibitor; indomethacin, non-selective COX-1/2 inhibitor; or acetaminophen (ATAP), a tentative COX-3 inhibitor. Intracisternal administration of either the vehicle or COX inhibitors did not affect formalin-induced scratching behavior. There were six animals in each group.

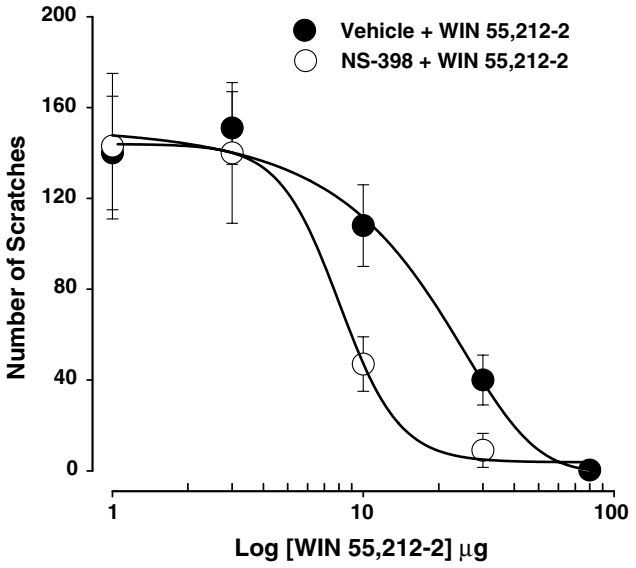

Fig. 7. Central administration of COX inhibitors causes an increased sensitivity to the cannabinoid agonist, WIN 55,212-2. Sigmoidal non-linear regression curve fitting for the dose–response data of WIN 55,212-2 and the estimation of ED_{50} of WIN 55,212-2 were investigated after pretreatment with NS-398, a selective COX-2 inhibitor. The ED_{50} value (7.4 ± 0.5 μg) of WIN 55,212-2 in the NS-398-treated group is significantly lower than the ED_{50} value (19.0 ± 3.7 μg) of WIN 55,212-2 in the vehicle-treated group. There were eight animals in each group.

($p < 0.05$, Fig. 7). The ED_{50} value (7.4 ± 0.5 μg) of WIN 55,212-2 in the NS-398-treated group is significantly lower than the ED_{50} value (19.0 ± 3.7 μg) of WIN 55,212-2 in the vehicle-treated group ($p < 0.05$).

4. Discussion

The present study is the first to demonstrate that central activation of cannabinoid receptor, CB1, reduces nociception induced by formalin injection in the TMJ and central blockade of COX pathways enhances the cannabinoid-induced antinociception. Although pain related to the TMJ is an important clinical problem, it is poorly understood due to the relative lack of experimental pain models. However, we (Ahn et al., 2005; Choi et al., 2005b) and others (Roveroni et al., 2001) have successfully used the nociceptive scratching behavior induced by formalin injection into the TMJ as a model system for studying modulation of TMJ-related pain. Measuring extravasation with Evans' blue dye confirms that intra-articular injection of formalin led to inflammation of the TMJ region that was not mimicked by injections outside the TMJ region.

Our findings that central administration of cannabinoids reduces the inflammatory nociception in the TMJ add to a growing awareness of cannabinoids as antinociceptive agents. Cannabinoids are known to inhibit the nociceptive transmission in the spinal cord, and the potency and efficacy of cannabinoids to produce antinociception are comparable to that of morphine (Buxbaum, 1972; Bloom et al., 1977). Although central administration of cannabinoids can produce antinociceptive effects, cannabinoids also produce profound motor effects such as immobility, catalepsy or hypolocomotion responses (Martin et al., 1991). The unwanted side effects associated with cannabinoids may limit the therapeutic potential (Johnson et al., 1981). The present study demonstrated that intracisternal administration of 10 or 30 μg of WIN 55,212-2 did not affect motor functions compared with the vehicle-treated rats. These results indicate that the intracisternal administration of WIN 55,212-2 reduced formalin-induced TMJ nociception and that the cannabinoid-induced suppression of nociception did not result from motor dysfunction. In the present study, we dissolved WIN 55,212-2 and other cannabinoid-related chemicals in 80% DMSO and 20% sterile saline. Although high concentrations of DMSO are associated with sedation and/or pain relieving properties (Sant and LaRock, 1994; Cavaletti et al., 2000), we demonstrated previously that intracisternal administration of vehicle (70% DMSO) alone did not alter the formalin-induced nociceptive behavior (Ahn et al., 2005). Consequently, we examined the effects of the vehicle (80% DMSO) on formalin-induced pain

behavior as a control. The vehicle (80% DMSO) administered alone did not alter the formalin-induced nociceptive behavior.

The present study also demonstrated that cannabinoid-induced antinociception in the TMJ region is mediated by the central activation of CB1 but not CB2 receptors. These observations are consistent with previous findings that demonstrated the role of peripheral CB2 receptors in mediating the antinociceptive action of peripherally administered cannabinoids (Malan et al., 2001; Scott et al., 2004) and central CB1 receptors in mediating the antinociceptive action of centrally administered cannabinoids (Scott et al., 2004). However, activation of CB2 receptor was also reported to inhibit the mechanical stimulus-evoked responses of WDR neurons in inflammatory and neuropathic pain models (Elmes et al., 2004).

Our studies also revealed that the effects of cannabinoids to reduce inflammatory pain in the TMJ region do not involve crossover utilization of opioid receptor pathways. This is important because pretreatment with naloxone, a broad spectrum opioid receptor antagonist, is reported to antagonize antinociception produced by intraperitoneal administration of Δ^9-THC or anandamide (Smith et al., 1998). Further, pretreatment with the kappa opioid receptor antagonist, nor-binaltorphimine, attenuates antinociception induced by Δ^9-THC in arthritic rats (Cox and Welch, 2004). These results indicate that endogenous opioid pathways can be involved in mediating the antinociceptive effects of cannabinoids. However, our findings showed that intracisternal pretreatment with naloxone did not affect cannabinoid-induced antinociception, suggesting that endogenous opioid pathways do not mediate the antinociceptive effects of WIN 55,212-2 in the inflammation-induced TMJ nociception.

Low doses (sub-threshold) of COX inhibitors were employed in order to demonstrate synergism with cannabinoid-induced antinociception. These low doses of COX inhibitors (30 μg) used in this study have not been shown previously to produce an analgesic effect in the model of formalin-induced nociception (Yamamoto and Nozaki-Taguchi, 2002; Ahn et al., 2005). Yamamoto and Nozaki-Taguchi (2002) previously demonstrated that an analgesic effect is produced at a higher dose of COX inhibitors (100 μg) but not at the lower dose (30 μg) we used in our study. Further, COX-2 and not COX-1 inhibitors were observed to produce an analgesic effect (Yamamoto and Nozaki-Taguchi, 2002). We demonstrated previously that central COX pathways participate in the IL-1β-induced hyperalgesia in the formalin-induced TMJ nociception (Ahn et al., 2005). Intracisternal administration of 30 μg of SC-560, NS-398, or acetaminophen did not affect the formalin-induced TMJ nociception but blocked the IL-1β-induced hyperalgesia. Low dose of COX inhibitors reduced the ED_{50} value of WIN 55,212. Administration of COX inhibitors or WIN 55,212 alone did not affect the formalin-induced nociceptive scratching behavior as compared with the vehicle-treated group. However, the combined administration of low dose of COX inhibitors and of WIN 55,212 produced antinociception.

Several different forms of COX have been characterized in previous studies. COX-1 is constitutively expressed in many somatic cell types and is considered to be a ''housekeeping'' enzyme that is involved in processes such as vascular hemostasis and gastroprotection (Katori and Majima, 2000). In contrast to COX-1, COX-2 is highly inducible in response to endotoxins, cytokines, growth factors, or other inflammatory stimuli (Kulkarni et al., 2000). Recently, COX-3 was discovered by Northern analysis of a canine cerebral cortex RNA using a COX-1 cDNA probe (Chandrasekharan et al., 2002) and COX-3 activity appears to be selectively inhibited by acetaminophen as well as a few of the antipyretic non-steroidal anti-inflammatory drugs (Botting, 2000). The mechanism of action of acetaminophen is far from settled and needs to be further clarified. Kis et al. (2005, 2006) demonstrated that the mouse COX-1b (COX-3) encodes a protein that has a completely different amino acid sequence than the known cyclooxygenases and lacks COX activity. Therefore, they argued that COX-1b is an unlikely target for acetaminophen (Kis et al., 2005, 2006). However, others have argued that the target of acetaminophen is the COX-3 enzyme that produces prostaglandins in the central nervous system (Botting, 2000; Ayoub et al., 2004; Botting and Ayoub, 2005). Although the present study demonstrated that acetaminophen was more potent than COX-2 inhibitor, it is not significant statistically. These results suggest that acetaminophen produced antinociceptive via pathways other than COX-1 and 2 pathways, ascertaining these pathways will require additional studies via multiple, complementary experimental strategies.

Interaction between COX pathways and cannabinoid has been tested in previous studies. The antinociceptive effect induced by indomethacin or flurbiprofen, COX inhibitors, was reversed by the co-administration of AM-251, a CB 1 antagonist (Guhring et al., 2002; Ates et al., 2003). Recently, the peripheral interactions between anandamide, an endocannabinoid, and ibuprofen, a non-specific COX inhibitor, have been demonstrated in the rat formalin test. Locally (hind paw) injected anandamide, ibuprofen or combined administration decreased pain behavior in the formalin test. The antinociceptive interaction between anandamide and ibuprofen was synergistic in peripheral tissue (Guindon et al., 2006). Our results indicate that central COX-2 and possibly putative COX-3 pathways play an important role in modulating the central antinociceptive effects of cannabinoids on TMJ nociception. These findings further suggest that a low dose of cannabinoids, together

with COX inhibitors, can reduce TMJ nociception. We have shown previously that central COX pathways participate in the transmission of nociception from oro-facial tissues including the TMJ (Choi et al., 2003c; Ahn et al., 2005). Our findings that central COX pathways modulate endocannabinoid-induced antinociception are consistent with findings in the hippocampus demonstrating the role of central COX pathways in modulating the effects of endocannabinoids (Kim and Alger, 2004).

In summary, central activation of CB1 receptors attenuated the nociceptive behavior induced by formalin injection in the TMJ. Further, central blockade of COX-2 pathways enhanced the antinociceptive effects mediated by activation of CB1 receptors. These results highlight the important therapeutic potential of combined administration of cannabinoids with COX inhibitors to effectively treat inflammatory pain associated with the temporomandibular joint.

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