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Low doses of cannabinoids enhance the antinociceptive effects of intracisternally-administered mGluRs groups II and III agonists in formalin-induced TMJ nociception in rats

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Abstract

The present study provides the first demonstration that central cannabinoids modulate the antinociceptive actions of metabotropic glutamate receptors (mGluRs) on formalin-induced temporomandibular joint (TMJ) nociception. Noxious scratching behavior induced by formalin injection in the TMJ was used as a model of pain. Intracisternal injection of 30 μ g of WIN 55,212-2, a non subtype selective cannabinoid receptor agonist, attenuated the number of scratches by 75% as compared with the vehicle-treated group, whereas vehicle alone or 3 or 10 μ g of WIN 55,212-2 had no effect. To explore the postulated interaction between central cannabinoid receptors and mGluRs, effects of combined administration of sub-analgesic doses of WIN 55,212-2 and group II or III mGluR agonists were tested. Group II or III mGluRs agonists were administered intracisternally 10 min after intracisternal administration of WIN 55,212-2. Neither 100 nmol APDC, a group II mGluRs agonist, nor L-AP4, a group III mGluR agonist, altered nociceptive behavior when given alone but significantly inhibited the formalin-induced nociceptive behavior in the presence of a sub-threshold dose (3 μ g) of WIN 55,212-2. The ED₅₀ value of APDC or L-AP4 was significantly reduced upon co-treatment with WIN 55,212-2, than in the vehicle-treated group, highlighting the important therapeutic potential of the combined administration of group II or III mGluR agonists with cannabinoids to effectively treat inflammatory pain associated with the TMJ. Potentiating effects of group II or III mGluRs agonists will likely permit administration of cannabinoids at doses that do not achieve significant accumulation to produce undesirable motor dysfunction.

Keywords

Antinociception; Cannabinoid; Formalin; mGluRs; TMJ

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1. Introduction

Glutamate produces its effects in the central nervous system by acting on multiple ionotropic and metabotropic receptors. The metabotropic glutamate receptors (mGluRs) are divided into three subgroups, based on their sequence similarities and transduction mechanisms [42,19, 45]. Group I receptors (mGluR1/5) are coupled to phospholipase C (PLC), while group II (mGluR 2/3) and group III receptors (mGluR 4/6-8) are negatively coupled to adenylate cyclase [38,39,44,37].

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 [30] and the spinal trigeminal nucleus [48], 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, a mGluR1/5 agonist [24].

Group II and III mGluRs also have been detected in the dorsal horn of the spinal cord [30, 50]. 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 [46]. Intrathecal administration of L-AP4, a group III mGluR agonist, also attenuated allodynia and neuronal responses in a model of neuropathic pain [12].

mGluRs immunoreactivity is localized in the trigeminal ganglion and in lamina II of the trigeminal subnucleus caudalis [5,49] regions that process nociceptive information from the orofacial area. We have previously demonstrated the involvement of trigeminal mGluRs in modulating nociceptive information originating from orofacial area by using APDC, a group II mGluR agonist, or L-AP4, a group III mGluR agonist, which reduced both IL-1 β -induced mechanical allodynia and mirror-image mechanical allodynia [32].

Recently, the participation of central cannabinoid receptors (CB) in modulating glutamate synaptic transmission has been demonstrated. Activation of CB1 receptors has been shown to suppress glutamatergic excitatory synaptic transmission in the hippocampus [47] and mediate tonic inhibition of glutamate release in the nucleus accumbens [53]. Interactions between mGluRs and cannabinoid receptors at both the spinal and supraspinal levels have also been shown. Intrathecal injection of MPEP, a selective mGluR5 antagonist, was shown to reverse the anti-hyperalgesic effect produced by the intrathecal application of a cannabinoid agonist [28]. Similarly, selective group II and III metabotropic receptor antagonists have been shown to block analgesia produced by cannabinoid microinjection into the PAG [40]. However, interactions between metabotropic receptors and cannabinoid receptors at the level of the trigeminal system remain unknown.

Our previous data demonstrated that an intracisternal injection of WIN 55,212-2, a non subtype selective cannabinoid (CB) receptor agonist, attenuated the number of scratches and the duration of scratching, respectively, and enhanced the antinociceptive effect produced by the blockade of the central COX pathways in temporomandibular joint (TMJ) nociception [2]. The present study extends those findings by addressing the hypothesis that central cannabinoid might modulate the antinociceptive roles of mGluRs in formalin-induced TMJ nociception. For this purpose, the antinociceptive effect of group II or III mGluR agonists, injected intracisternally, was tested after an intracisternal pretreatment with the cannabinoid agonist, WIN 55,212-2.

2. Materials and Methods

2.1. Animals

Experiments were carried out on 344 male Sprague-Dawley rats (Taconic, eight animals per experimental group) weighing 220-280 g. The animals were maintained in a temperature-controlled room ($23 \pm 1^\circ\text{C}$) with a 12/12 hr light-dark cycle (light on at 7:00A.M.). All procedures involving the use of the 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. Intra-articular injection of formalin into the TMJ

Each animal was first placed in a Plexiglas box for a 30 min period to minimize stress [1,43]. Rats were not allowed access to food or water during the test. After the acclimation period, each animal was removed from the test chamber and anesthetized by the inhalation of 5% halothane to allow for the TMJ injection. Formalin was injected in the TMJ region as described previously [1,2,15], via a 30-gauge needle introduced into the capsule of the left TMJ. A cannula consisting of a polyethylene tube was connected to the needle and to a Hamilton syringe (50 μL) previously with the 5% of formalin solution. The volume of the TMJ injections was 50 μL . Following from the anesthesia and was returned to the test chamber for a 45 min observation period. For each animal, the number of noxious behavioral responses, such as grooming, rubbing, and/or scratching the TMJ region, was recorded for 9 successive 5-min intervals [1,43]. Behavioral responses induced by the formalin injection in the TMJ did not display the two distinct phases [13,14,16,17,18] because the early phase was partly masked by the anesthesia. Therefore, we analyzed the total number of scratches in the second phase (11-45 min, 2nd phase) as indices of TMJ nociception after the formalin injection, as described previously [1,2,15,43].

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 [1,15,43]. The same volume of formalin was injected into the right masseter muscle. In a separate series of experiments, saline was injected into the TMJ region as a control.

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

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 [1,3,32,52]. 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. After a 72-hour recovery period from surgery, WIN 55,212-2 (3, 10 or 30 $\mu\text{g}/10 \mu\text{L}$) was administered intracisternally through the implanted PE tube 20 min prior to the injection of formalin in freely moving rats. In the control group, 10 μL of the vehicle (40% of (2-hydroxypropyl)-beta-cyclodextrin solution) of WIN 55,212-2 was injected intracisternally. Because intrathecal catheterization may produce motor dysfunction, we examined whether or not an intracisternal catheter produces motor dysfunction. After implantation of an intracisternal catheter, only animals that displayed normal motor functions were evaluated. For confirmation of the placement of the intracisternal cannula and the extent of the spread of the drugs, pontamine sky blue dye was injected at the end of the tests. In the present study, the animals that showed motor dysfunction or mal-position of the catheter after intracisternal catheterization were excluded from analysis.

2.4. Effects of the combined administration of WIN 55,212-2 and groups II or III mGluRs agonists on the formalin-induced TMJ nociception

In order to investigate the interaction between cannabinoid receptors and central mGluRs, group II or III mGluRs agonists and WIN 55212-2 were administered intracisternally at sub-analgesic doses. APDC [(2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate, 100 nmol/ 10 μ L], a selective group II mGluR agonist, or L-AP4 [l-(+)-2-amino-4-phosphonobutyric acid, 30 nmol/ 10 μ L], a selective group III mGluR agonist, was intracisternally administered, respectively. Vehicle or WIN 55,212-2 (3 μ g/ 10 μ L) was administered through the cisternal catheter 10 min prior to the intracisternal administration of APDC and L-AP4. We also examined the effects of LY 341495 [(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid, 0.5 nmol/ 10 μ L], a group II mGluR antagonist, or CPPG [(RS)-phosphonopentanoic acid, 75 nmol/ 10 μ L], a group III mGluR antagonist, after intracisternal administration 20 min prior to the intracisternal injection of APDC or L-AP4, respectively. Fig 1 illustrates timing of various microinjections.

We investigated the dose-dependent antinociceptive effects of APDC and L-AP4 in the 3 μ g of WIN 55,212-2-treated animals. We injected intracisternally APDC (4, 10, 30, 100 or 300 nmol) and L-AP 4 (10, 30, 100, 300 or 1000 nmol) after the intracisternal administration of 3 μ g of WIN 55,212-2 or vehicle. Data analysis involved a sigmoidal non-linear regression curve fitting for the dose-response data and the estimation of ED₅₀ for APDC and L-AP4.

2.5. Verification of inflammation

To confirm that the plasma extravasation induced by the TMJ injection was indeed restricted to the TMJ region, we measured formalin-induced plasma extravasation of Evans' blue dye bound to the plasma protein, as described previously [1,2,10,15,27,29]. 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 left 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 the absorbance at 620 nm. The recovery of the extravasated dye per gram weight of tissue (μ g/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.

2.6. Rotarod test

Changes in motor performance, after the intracisternal administration of 3, 10, or 30 μ g of WIN 55,212-2, were measured using a rotarod (Ugo Basil, Comerio), as described previously [2, 7]. The rotarod speed increased to 16 rpm over a 180-seconds period, with the maximum time spent on the rod set at 300 seconds. Rats received two or three training trials, on two separate days prior to testing, for acclimatization. On the experimental day, the time course of motor performance was examined before and after intracisternal administration of WIN 55,212-2.

2.7 Chemicals

WIN 55,212-2 was dissolved in a (2-hydroxypropyl)-beta-cyclodextrin solution (45% (w/v), Sigma). APDC and L-AP4 were dissolved in normal saline. A stock solution of 0.1 mM of LY341495, a group II mGluR antagonist, and CPPG, a group III mGluR antagonist, was made in 1.2 eq of NaOH. LY341495 (0.5 nmol/10 μ L) and CPPG (75 nmol/10 μ L) were diluted in normal saline. WIN 55,212-2 and all mGluR-related chemicals were obtained from Tocris-

Cookson. Intracisternal injections were made through an implanted PE tube. The PE tube had a dead space volume of 7 μ L and 8 μ L of saline was injected to flush the cannula following each microinjection. In the control group, the vehicle for APDC and L-AP4 (saline), LY341495 and CPPG (NaOH vehicle), or WIN 55,212-2 (45% 2-hydroxypropyl)-beta-cyclodextrin solution) was injected intracisternally for comparison of drug-modulated versus control responses.

2.8. Statistical analysis

Statistical analysis of the behavioral data was carried out with a one-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc analysis. The dose-dependent curves were compared using two-way ANOVA and a Bonferroni post-hoc analysis. $P < 0.05$ was considered to be statistically significant. All data are presented as mean $\pm$ SEM. ED₅₀ values were determined for each agonist using the methods of non-linear regression curve fitting: $I = I_{max} - I_{max} \times [C^n / (C^n + IC_{50}^n)]$, where I is the inhibitory effect of APDC or L-AP4, C is the concentration of APDC or L-AP 4, IC_{50} is the molar concentration of APDC or L-AP4 that produced 50% of the maximum possible effect and n is the Hill coefficient. ED50 values are reported with 95% confidence interval limits (CI).

3. Results

Intra-articular injection of formalin-induced TMJ nociception

The average concentration of extravasated Evans' blue dye in tissues obtained from the TMJ is illustrated in Fig. 2 (left). The level of dye was significantly higher in the formalin-treated group ($16.3 \pm 3.5 \mu\text{g}$, $p < 0.05$) as compared to the vehicle (saline)-treated group ($3.9 \pm 1.0 \mu\text{g}$). However, the amount of dye on the contralateral TMJ side did not differ from that of the saline-treated group. We observed repeatedly that injection of Evans' blue dye into the TMJ cavity did not spread to surrounding tissues. Moreover, an intra-muscular injection of formalin did not increase the amount of Evans' blue dye in the TMJ. Intra-articular injection of 50 μ L of 5% formalin significantly produced noxious scratching behavioral responses that lasted for 45 min (Fig. 2, right). Intra-articular administration of formalin produced a total of 149 ± 22 scratches ($p < 0.05$) in the second phase (11-45 min) while it did not affect the scratching behavior in the first phase (0-10 min), as compared with the vehicle-treated or naïve rats.

Effects of the intracisternal injection of WIN 55,212-2 on nociceptive scratching behavior induced by the formalin injection

In order to determine whether cannabinoids modulate formalin-induced TMJ nociception, the effects of an intracisternally-administered cannabinoid agonist were tested on the nociceptive behavior induced by the formalin injection in the TMJ. Fig. 3 illustrates the antinociceptive effect of the intracisternally-administered WIN 55,212-2, a non subtype selective CB1/2 receptor agonist, on the number of scratches produced by the formalin injection into the TMJ region. Neither the vehicle nor 3 μg dose of WIN 55,212-2 altered the formalin-induced scratching behavior. Although an intracisternal injection of 10 μg of WIN 55,212-2 attenuated the number of scratches, this attenuation is not statistically significant. However, an intracisternal injection of 30 μg of WIN 55,212-2 attenuated the number of scratches by 75% (34 ± 10 in the number of scratches, $p < 0.05$), as compared with the vehicle-treated group. Although intracisternal administration of 3 or 10 μg of WIN 55,212-2 did not affect any motor functions compared with the vehicle-treated rats, 30 μg dose of WIN 55,212-2 produced a slight but non-significant motor impairment (data not shown.).

Low dose of cannabinoid enhanced antinociceptive effects of intracisternally-administered groups II and III mGluRs agonists

In order to understand the interaction between central cannabinoid receptors and mGluRs in modulating inflammatory TMJ nociception, the effects of the combined administration of WIN 55,212-2 and group II or III mGluR agonists were tested (Fig. 4 and 5). Intracisternal administration of 3 μ g of WIN 55,212-2 or 100 nmol of APDC did not affect the formalin-induced TMJ nociceptive behavior. Intracisternal administration of a sub-analgesic dose of APDC (100 nmol), a group II mGluR agonist, significantly reduced the formalin-induced nociceptive behavior in the presence of 3 μ g of WIN 55,212-2. The antinociceptive action of APDC in the WIN 55,212-2-treated group was blocked by a pretreatment with 0.5 nmol of LY341495, a group II mGluR antagonist (Fig. 4).

Fig. 5 illustrates the effects of the combined administration of L-AP4, a group III mGluR agonist, and WIN 55,212-2 on the formalin-induced TMJ scratching behavior. Neither pretreatment with 30 nmol of L-AP4 nor 3 μ g of WIN 55,212-2 altered the formalin-induced scratching behavior in the TMJ regions. However, combined administration of 30 nmol of L-AP4 and 3 μ g of WIN 55,212-2 significantly reduced the number of scratches induced by intra-articular injection of formalin, as compared with the vehicle-treated group. Antinociception, produced by the combined administration of 30 nmol of L-AP4 and 3 μ g of WIN 55,212-2, was blocked by pretreatment with 75 nmol of CPPG, a group III mGluRs antagonist.

We compared the dose-response curves for the antinociceptive effects of group II (Fig. 6) or group III (Fig. 7) mGluRs in the WIN 55,212-2 or vehicle-treated groups. Intracisternal administration of APDC significantly reduced number of scratches produced by intra-articular formalin injection in a dose-dependent manner ($F_{(4,70)}=8.761$, $p<0.05$). Pretreatment with a sub-analgesic dose of WIN 55,212-2 (3 μ g) significantly enhanced the antinociceptive effects of APDC as compared with that of the vehicle-treated group ($F_{(1,70)}=4.762$, $p<0.05$) (Fig. 6). The ED_{50} value of APDC [32 nmol (95% CI 23.18 - 40.82 nmol; $n=40$)] in the WIN 55,212-2-treated group shifted to the left as compared with the ED_{50} value of APDC [150 nmol (95% CI 123.15 - 178.85 nmol; $n=40$)] in the vehicle-treated group. The 95% CI did not overlap between groups. Intracisternal administration of L-AP4 also produced antinociceptive effects in a dose-dependent manner ($F_{(4,70)}=12.688$, $p<0.05$) and significantly enhanced the antinociceptive effects of L-AP4 in the sub-analgesic dose of WIN 55,212-2 (3 μ g)-treated group as compared with that of the vehicle-treated group ($F_{(1,70)}=6.722$, $p<0.05$) (Fig 7). Pretreatment with a sub-analgesic dose of WIN 55,212-2 also attenuated the ED_{50} value of L-AP4 from 101 nmol (95% CI 81.99 - 120.01 nmol; $n=40$) to 25 nmol (95% CI 18.14 - 31.86 nmol; $n=40$) with no overlap between 95% CI of two groups.

4. Discussion

The present study is the first to demonstrate that the central activation of a cannabinoid receptor enhances antinociception produced by intracisternally-administered groups II or III mGluRs agonists in a model of TMJ nociception. Intracisternal administration of sub-analgesic doses of APDC (100 nmol) or L-AP4 (30 nmol) produced antinociceptive action in the presence of a sub-analgesic dose (3 μ g) of WIN 55,212-2. Moreover, antinociceptive effects produced by the combined administration of sub-analgesic doses of APDC (100 nmol) or L-AP4 (30 nmol) and sub-analgesic doses of WIN 55,212-2 were blocked by pretreatment with LY341495, a group II mGluR antagonist, or CPPG, a group III mGluR antagonist. These results are consistent with the interpretation that the enhanced antinociceptive effect is principally due to potentiation mediated through central cannabinoid receptors and central groups II or III mGluRs. Our findings provide new therapeutic value for treating pain disorders without the motor dysfunction induced by cannabinoids.

Recent studies have demonstrated an inter-relationship between cannabinoid receptors and mGluRs in the central nervous system. Group I mGluRs are known to modulate endocannabinoid-mediated inhibition of GABAergic inputs on Purkinje cells [26] and inhibitory postsynaptic current activated by cannabinoid receptors in pyramidal cells [51]. Glutamatergic excitatory synaptic transmission is suppressed by activation of CB1 receptors in the hippocampus [47] and Purkinje cells [34]. Group II or III mGluRs also participate in the long-term or short-term plasticity with direct and indirect interactions between CB1 receptors and mGluRs in pyramidal neurons from the prefrontal cortex [6]. Further, CB1 receptors exert a tonic inhibition of glutamate release and these effects appear to be mediated by the activation of presynaptic mGluR2/3 autoreceptors in the nucleus accumbens [53].

The interactions between group I mGluRs and cannabinoid receptors in nociceptive processing have been shown in several previous studies. Systemic injection of the synthetic cannabinoid agonist WIN55, 212-2 increased withdrawal thresholds of hind paw in rats with a peripheral nerve injury and intrathecal injection of MPEP, a selective mGlu5 glutamate receptor antagonist, reversed the antihyperalgesic effect of intrathecal WIN55,212-2 [28]. Central injection of MPEP in the PAG prevented the WIN-induced inhibition of neuronal response in the RVM [20].

The possible contribution of group II and III mGluRs to cannabinoid-induced antinociception has been studied at supraspinal sites in rats [40]. Pretreatment with either 2-(S)-alpha-EGlu or (RS)-alpha-MSOP, selective antagonists for group II and III mGluRs, respectively, prevented the analgesia produced by microinjection of WIN 55,212-2 in the PAG. Although the participation of central cannabinoid receptors in modulating glutamate synaptic transmission has been demonstrated, the present studies provide the first direct evidence of interactions between central cannabinoid receptors and group II or III mGluRs in modulation TMJ nociception.

Although the present study demonstrated that low dose of WIN55,212-2 enhanced antinociception produced by intracisternally-administered groups II or III mGluRs agonists, the underlying cellular mechanisms remain largely unknown. CB1 agonists have been demonstrated to inhibit the synaptic release of a variety of neurotransmitters, including glutamate and γ -aminobutyric acid (GABA), as well as norepinephrine, acetylcholine, dopamine and glycine in CNS [21]. The group II and III mGluRs are known to be expressed at presynaptic sites on glutamatergic terminals and act either to limit calcium entry through voltage-gated calcium channels or through a direct disruption of vesicle-release machinery. These results suggest that one of the functions of group II and III mGluRs is to inhibit glutamate release through presynaptic autoreceptors at central synapses [4,19,21]. Moreover, excitatory synaptic transmission in forebrain areas was directly modulated by cannabinoid receptors that are expressed on presynaptic axon terminals originating from glutamatergic neurons [22]. These results, taken together with the present data, indicate a possible interaction between mGluRs and cannabinoid receptors in presynaptic terminals.

Our findings demonstrate that the central administration of cannabinoids reduces inflammatory nociception in the TMJ. Cannabinoids are known to inhibit nociceptive transmission in the spinal cord, and the potency and efficacy of cannabinoids to produce antinociception are comparable to that of morphine [8,9]. The intrathecal administration of cannabinoid receptor agonists produces antinociception and reduces hypersensitivity in neuropathic and inflammatory pain models [25,36]. Electrophysiological studies have also demonstrated that cannabinoid receptor agonists inhibit nociceptive neuronal activity in the dorsal horn of the lumbar spinal cord [11,23,33] and in the spinal trigeminal nucleus caudalis [41]. Although the central administration of cannabinoids can produce antinociceptive effects, cannabinoids also produce profound motor effects such as immobility, catalepsy or hypolocomotion responses

[35]. The unwanted motor dysfunctions associated with cannabinoids may limit their therapeutic potential [31]. However, the intracisternal administration of 3 or 10 µg of WIN 55,212-2 did not affect motor functions compared with the vehicle-treated rats in the present investigation. These results indicate that the enhancement of antinociceptive effects of produced by intracisternal administration of sub-analgesic doses of group II or III mGluRs agonists in the WIN 55,212-2-treated animals did not result from cannabinoid-induced motor dysfunction.

In summary, the central activation of cannabinoid receptors attenuated the nociceptive behavior induced by formalin injection in the TMJ. Further, pretreatment with a low dose of cannabinoid enhanced antinociceptive effects of intracisternally-administered group II or III mGluR agonists. These results highlight the important therapeutic potential of the combined administration of sub-analgesic doses of group II or III mGluRs agonists with a sub-analgesic doses of cannabinoid to effectively treat inflammatory pain associated with the temporomandibular joint. Further, potentiating effects of group II or III mGluRs agonists will permit the administration of cannabinoids at doses that do not achieve any significant accumulation to produce undesirable motor dysfunction.

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Fig 1.
Line drawing indicates timing of various microinjections.

Fig. 2.

Intra-articular injection of formalin-induced TMJ nociception. Measurement of the location of the inflammatory response based on assessment of the plasma protein extravasation using Evans' blue dye (left panel) and the number of scratches was measured for 9 successive 5-min intervals (Right panel). Vehicle-TMJ, saline injected into TMJ; Formalin-TMJ, formalin injected into TMJ; Formalin-masseter muscle, formalin injected into the masseter muscle; Formalin-contralateral TMJ, dye concentration of contralateral TMJ. There were eight animals in each group. * $P < 0.05$, Vehicle- vs. Formalin-treated group.

Fig. 3.

Intracisternal administration of WIN 55,212-2 (30 µg), a non subtype selective CB1/2 receptor agonist, reduced the number of scratches produced by an intra-articular injection of formalin into TMJ. WIN 55,212-2 (3, 10, or 30 µg) was injected 20 min prior to the formalin injection. There were eight animals in each group. * $P < 0.05$, Vehicle vs. WIN 55,212-2-treated group.

Fig. 4.

Intracisternal administration of a sub-analgesic dose of APDC, a group II mGluRs receptor agonist, produced an antinociceptive effect in the presence of a sub-threshold dose of WIN 55,212-2. Neither intracisternal administration of 3 µg of WIN55,212-2 nor 100 nmol of APDC alone produced antinociception. There were eight animals in each group. * $P < 0.05$, Vehicle + APDC + Formalin vs. WIN55,212-2 + APDC + Formalin. # $P < 0.05$, Vehicle + WIN55,212-2 + APDC + Formalin vs. LY341495 + WIN55,212-2 + APDC + Formalin

Fig. 5.

Intracisternal administration of a sub-analgesic dose of L-AP4, a group III mGluRs receptor agonist, produced antinociceptive effects in the presence of a sub-threshold dose of WIN 55,212-2. Neither intracisternal administration of 3 µg of WIN55,212-2 nor 30 nmol of L-AP4 alone produced antinociception. There were eight animals in each group. * $P < 0.05$, Vehicle + L-AP4 + Formalin vs. WIN55,212-2 + L-AP4 + Formalin. # $P < 0.05$, Vehicle + WIN55,212-2 + L-AP4 + Formalin vs. CPPG + WIN55,212-2 + L-AP4 + Formalin

Fig. 6.

Intracisternal pretreatment with sub-analgesic doses of WIN 55,212-2 enhances the antinociceptive effects of the central group II mGluRs agonist. Sigmoidal non-linear regression curve fitting for the dose-response data of APDC, group II mGluRs agonist, and the estimation of ED₅₀ of APDC were investigated in the 3 µg of WIN 55,212-2-treated animals. The ED₅₀ value [32 nmol (95% CI 23.18 - 40.82 nmol; n=40)] of APDC in the WIN 55,212-2-treated group shifted to the left as compared with the ED₅₀ value [150 nmol (95% CI 123.15 - 178.85 nmol; n=40)] of APDC in the vehicle-treated group.

Fig. 7.

Intracisternal pretreatment with sub-analgesic doses of WIN 55,212-2 enhanced antinociceptive effects of the central group III mGluRs agonist. Sigmoidal non-linear regression curve fitting for the dose-response data of L-AP4, group III mGluRs agonist, and the estimation of ED₅₀ of L-AP4 were investigated in the 3 µg of WIN 55,212-2-treated group. The ED₅₀ value [25 nmol (95% CI 18.14 - 31.86 nmol; n=40)] of L-AP4 in the WIN 55,212-2-treated group shifted to the left as compared with the ED₅₀ value [101 nmol (95% CI 81.99 - 120.01 nmol; n=40)] of L-AP4 in the vehicle-treated group.