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# Spinal Cord is the Primary Site of Action of the Cannabinoid $CB_2$ Receptor Agonist JWH133 that Suppresses Neuropathic Pain: Possible Involvement of Microglia

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**Abstract:** Neuropathic pain, a highly debilitating condition that commonly occurs after damage to the nervous system, is often resistant to commonly used analgesic agents such as non-steroidal anti-inflammatory drugs and even opioids. Several studies using rodent models reported that cannabinoid $CB_2$ receptor ($CB_2R$) agonists are effective for treating chronic pain. However, the analgesic mechanism of $CB_2R$ agonists in neuropathic pain states is not fully understood. In this study, we investigated the role of $CB_2Rs$ in the development and maintenance phases of neuropathic pain, and the mechanism of the $CB_2R$-mediated analgesic effect on neuropathic pain. In a rat model of neuropathic pain, systemic administration of JWH133, a $CB_2R$ agonist, markedly improved tactile allodynia, and this effect was prevented by intrathecal pretreatment with AM630, a $CB_2R$ antagonist. The antiallodynic effect of intrathecally administered JWH133 was inhibited by intrathecal pretreatment with pertussis toxin or forskolin. In the spinal cord, $CB_2R$ expression was significantly increased on post-operative day 3, and persisted for 2 weeks. Furthermore, repeated intrathecal administration of JWH133 notably attenuated the development of tactile allodynia after peripheral nerve injury. In a culture of microglia activated by overexpressing interferon regulatory factor 8, a transcription factor crucial for neuropathic pain, JWH133 treatment suppressed the increased expression of interleukin-1β. Our findings suggest that activation of $CB_2Rs$ upregulated in the spinal cord after nerve injury alleviates existing tactile allodynia through the $G_{i/o}$-adenylate cyclase signaling pathway and suppresses the development of allodynia. This process may reduce the inflammatory response of microglia. Therefore, spinal $CB_2Rs$ may be a therapeutic target for the treatment of neuropathic pain.

**Keywords:** Allodynia, cannabinoid $CB_2$ receptor, microglia, neuropathic pain, spinal cord.

## INTRODUCTION

Neuropathic pain is a highly debilitating chronic pain state that occurs after nerve damage associated with various diseases, such as cancer, diabetes mellitus, infection, autoimmune disease, and trauma. Tactile allodynia (pain hypersensitivity to normally innocuous stimuli) is one of the characteristic symptoms of neuropathic pain and is often refractory to currently available treatments such as non-steroidal anti-inflammatory drugs (NSAIDs), and even opioids. Therefore, new therapeutic agents with higher efficacy for the treatment of neuropathic pain are desirable.

Accumulating evidence from diverse animal models of neuropathic pain indicates that neuropathic pain is a reflection of the aberrant excitability of dorsal horn neurons evoked by peripheral sensory inputs [1, 2]. This hyperexcitability might result from multiple cellular and molecular alterations in the dorsal horn occurring after peripheral nerve injury (PNI). It has long been considered that there are damage-related changes in neurons, but recent studies provide compelling evidence indicating that spinal microglia, immune-like glial cells in the central nervous system (CNS), rapidly respond to PNI and become activated with changing morphology, increased numbers, and expression of a variety of genes [3, 4]. Activated spinal microglia secrete various biologically active signaling molecules, including proinflammatory cytokines [5], which induce hyperexcitability of dorsal horn neurons [4, 6]. Thus, it is important to control the activation of microglia for the treatment of neuropathic pain.

Recently, cannabinoids have been shown to have potent analgesic effects in studies using several chronic pain models (including neuropathic pain) [7, 8]. For the treatment of chronic pain, cannabinoids have attracted much attention as potential new therapeutic alternatives to opioids. Cannabinoids activate their cognate receptors $CB_1$, $CB_2$, and GPR55 [9-11]. Among these cannabinoid receptors, the $CB_2$ receptor ($CB_2R$) may be a target for the development of novel analgesics because $CB_2R$ agonists have been reported to exert their antinociceptive effects without causing neuropsychiatric side effects [8, 12, 13]. $CB_2Rs$ are mostly expressed in peripheral tissues as well as immune cells, where they regulate cell activation and cytokine release [14-16]. In normal rats, systemic administration of AM1241, a $CB_2R$ agonist, produces an antinociceptive effect, which can

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be prevented by intrapaw injection of naloxone [17]. These results suggest that $CB_2R$ activation stimulates the release of β-endorphin from keratinocytes, which acts at local neuronal μ-opioid receptors to inhibit nociception. Under neuropathic pain conditions, intraplantar injection of the $CB_2R$ agonist has no effect, but intrathecal administration produces an anti-allodynic effect [18]. Furthermore, $CB_2Rs$ are expressed by activated microglia [19-21]. Thus, the mechanism of $CB_2R$-mediated pain inhibition might be different between normal and neuropathic conditions. However, it remains to be determined where the primary site of action of $CB_2R$ agonists is when administered systemically to suppress neuropathic pain.

To address these issues, we investigated the antiallodynic effect of a $CB_2R$ agonist administered systemically, the intracellular signaling pathway downstream of $CB_2R$, and the contribution of $CB_2R$ to the development and maintenance of neuropathic pain. Furthermore, using cultured microglial cells activated by overexpressing interferon regulatory factor 8 (IRF8), a molecule previously identified as a transcription factor of the IRF family and crucial for microglial activation after PNI and neuropathic pain [22], we investigated the effect of a $CB_2R$ agonist on IRF8-induced gene expression.

## MATERIALS AND METHODOLOGY

### Animals

Male Wistar rats (230–280 g) obtained from Japan SLC (Hamamatsu, Japan) were used. Rats were housed at a constant room temperature of 23 ± 1°C with a 12-h light-dark cycle (light on 8:00–20:00) and fed food and water *ad libitum*. All of the animals used in the present study were treated in accordance with the guidelines of Kyushu University.

### Neuropathic and Inflammatory Pain Model

Preparation of both chronic pain models of rats was performed under isoflurane (2%) anesthesia. For the neuropathic pain model, a unilateral L5 spinal nerve of rats was tightly ligated with 5-0 silk suture and cut just distal to the ligature. For the inflammatory pain model, complete Freund's adjuvant (CFA; 0.1 mg/200 μL, suspended in an equal volume of phosphate-buffered saline (PBS); Sigma, St. Louis, MO, USA) was injected into the plantar surface of the left hindpaw of rats.

### Behavioral Assays

To assess tactile allodynia, rats were placed individually in a wire mesh cage and habituated for 30 min to allow acclimatization to the new environment. Then, calibrated von Frey filaments (0.4–15.0 g; North Coast Medical, Morgan Hill, CA, USA) were applied to the plantar surface of the hindpaw from below the mesh floor. Paw withdrawal threshold (PWT) was determined using the up-down method [23].

### Intrathecal Catheterization

Under isoflurane (2%) anesthesia, rats were implanted with a 32-gauge catheter (ReCathCo, Allison Park, PA, USA) through the atlanto-occipital region and into the lumbar enlargement (close to the L4–5 segments) of the spinal cord [24]. After 3 days of recovery, the catheter placement was verified by the observation of transient hindpaw paralysis induced by intrathecal injection of lidocaine (2%, 7 μL). Animals that failed to show any paralysis were not used in experiments.

### Drug Administration

To examine the acute effect, vehicle [PBS/1% dimethyl sulfoxide (DMSO)] or JWH133 (1 and 3 mg/kg or 0.1, 1, and 10 nmol in 10 μL PBS/1% DMSO; Tocris Bioscience, Bristol, UK) were administered intraperitoneally or intrathecally, through the implanted catheter, to nerve-injured rats 7 days after PNI. AM630 (20 nmol in 10 μL PBS/1% DMSO/1% Tween 80; Tocris Bioscience, Bristol, UK), pertussis toxin (PTx; 0.5 μg in 10 μL PBS; Sigma) and forskolin (10 μg in 10 μL PBS/1% DMSO; Sigma) were administered by intrathecal or intraplantar injection before JWH133 administration. The drug was administered just after pre-measurement of PWT. To examine the chronic effect, rats were administered vehicle or JWH133 intrathecally twice a day from day 0 (immediately after nerve injury) to 7 or from day 7 to 14. PWT was measured just before morning drug administration (at least 10 h after drug administration of the previous day).

### Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Rats were deeply anesthetized intraperitoneally with pentobarbital (100 mg/kg), perfused transcardially with PBS, and the L5 segment of the spinal cord without L5 roots was removed immediately. The tissue was vertically separated at the median, and hemisections of the spinal cord were subjected to total RNA extraction using TRIsure (Bioline, Danwon-gu, South Korea) according to the manufacturer's protocol and purified with an RNeasy mini-plus kit (Qiagen, Valencia, CA, USA). Extraction of total RNA from a BV-2 mouse microglial-cell line was also performed using TRIsure. The amount of total RNA was measured using a Nanodrop spectrophotometer (Nanodrop, Wilmington, DE, USA). Complementary DNA was prepared *via* reverse transcription of total RNA using Prime Script reverse transcriptase (Takara, Kyoto, Japan). Quantitative PCR was performed with Premix Ex Taq (Takara) using a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Expression levels were normalized to the values for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The TaqMan probe and the forward and reverse primers used in this study are shown in Table **1**. The primers and probe for GAPDH were obtained from Applied Biosystems.

### Cell Culture

The BV-2 mouse microglial cell line was cultured as described previously [25]. Briefly, cells were cultured in Dulbecco's modified Eagle's medium with 5% heat-inactivated fetal bovine serum, 2 mM L-glutamine, penicillin, and streptomycin. The lentiviral vector was prepared as described previously to overexpress the IRF8 gene [22]. In brief, full-length cDNAs for mouse IRF8 tagged with green fluorescent protein (IRF8-GFP) or GFP

**Table 1. Sequences of forward primers, reverse primers, and TaqMan probes for quantitative real-time RT-PCR.**

| | Primer | | Probe |
|---|---|---|---|
| rCB2R | Forward | 5′-CATGCTGTCTCTGGCAGATTCA-3′ | 5′-FAM-CCAACAGACAAGACGTCCGTCCCC-TAMRA-3′ |
| | Reverse | 5′-ATTAGGGAGGCTGATTGGTCTTCT-3′ | |
| mCB2R | Forward | 5′-GGATGCCGGGAGACAGAA-3′ | 5′-FAM-CCAACGGCTCCAACGGTGGCTT-TAMRA-3′ |
| | Reverse | 5′-TGCTCAGGATCATGTACTCCTTCA-3′ | |
| mIRF8 | Forward | 5′-GGATATGCCGCCTATGACACA-3′ | 5′-FAM-CCATTCAGCTTTCTCCCAGATGGTCATC-TAMRA-3′ |
| | Reverse | 5′-CATCCGGCCCATACAACTTAG-3′ | |
| mP2X4R | Forward | 5′-ACAACGTGTCTCCTGGCTACAAT-3′ | 5′-FAM-CAATGAGCAACGCACACTCACCAAGG-TAMRA-3′ |
| | Reverse | 5′-GTCAAACTTGCCAGCCTTTCC-3′ | |
| mP2Y12R | Forward | 5′-TGAAGACCACCAGGCCATTT-3′ | 5′-FAM-AAACGTCCAGCCCCAGCAATCTCTTG-TAMRA-3′ |
| | Reverse | 5′-AGGCCCAGATGACAACAGAAA-3′ | |
| mTNF-α | Forward | 5′-GTTCTCTTCAAGGGACAAGGCTG-3′ | 5′-FAM-TACGTGCTCCTCACCCACACCGTCA-TAMRA-3′ |
| | Reverse | 5′-TCCTGGTATGAGATAGCAAATCGG-3′ | |
| mIL-1β | Forward | 5′-GAAAGACGGCACACCCACC-3′ | 5′-FAM-TGCAGCTGGAGAGTGTGGATCCCAA-TAMRA-3′ |
| | Reverse | 5′-AGACAAACCGCTTTTCCATCTTC-3′ | |
| mIL-6 | Forward | 5′-GGGACTGATGCTGGTGACAA-3′ | 5′-FAM-TCACAGAGGATACCACTCCCAACAGACCTG-TAMRA-3′ |
| | Reverse | 5′-TGCCATTGCACAACTCTTTTCT-3′ | |

r, rat; m, mouse; CB2R, cannabinoid receptor 2; IRF, interferon regulatory factor; P2X4R, P2X purinoceptor 4; P2Y12R, P2Y12 receptor; TNF, tumor necrosis factor; IL, interleukin.

alone were cloned into the lentiviral CS2-EF-MCS vector (RIKEN, Saitama, Japan). Each vector with pCAG-HIVgp (packaging plasmid; RIKEN) and pCMV-VSV-G-RSV-Rev (RIKEN) was co-transfected into HEK293T cells. After mixing with polyethylene glycol, viral particles and polybrene were added onto BV-2 microglial cells plated on 24-well plates. After a 12-h treatment with the lentivirus, culture medium was changed to new medium, and cells were cultured further for 60 h. For the gene expression experiments, the transduced cells were subjected to total RNA extraction as described above.

### cAMP Assay

BV-2 cells were seeded in 24-well plates. After treatment with reagents, the intracellular concentration of cAMP was estimated using an Amersham cAMP Biotrak Enzymeimmunoassay system (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's protocol.

### Statistical Analysis

All data were presented as the mean ± SEM. The statistical significance of differences between the values was evaluated by the Student's *t*-test, one-way analysis of variance (ANOVA) with the Dunnett's *post hoc* test, or two-way ANOVA with the Bonferroni's *post hoc* test. Differences were considered significant when the p-value was $<0.05$.

## RESULTS

### Primary Site of Action of JWH133 on Tactile Allodynia Induced by PNI

Consistent with our previous studies [24], the PWT of the ipsilateral hindpaw to PNI in rats markedly decreased. The peak of threshold decrease was observed on days 7 or 14 after PNI (data not shown). On day 7, we intraperitoneally administered JWH133 (3 mg/kg), a selective $CB_2R$ agonist, and found that JWH133 remarkably alleviated tactile allodynia (Fig. **1A**). The effect of JWH133 occurred in a dose-dependent manner (Fig. **1B**). To determine the site of action of the JWH133-induced antiallodynic effect, we intrathecally administered the selective $CB_2R$ antagonist AM630 (20 nmol) prior to intraperitoneal (i.p.) administration of JWH133. AM630 completely prevented the antiallodynic effect of JWH133 (Fig. **1C**). In contrast, the JWH133 (i.p.)-induced antiallodynic effect was not affected by intraplantar pretreatment with AM630 (Fig. **1C**). Furthermore, intrathecal administration of JWH133 produced an antiallodynic effect in a dose-dependent manner (Fig. **1D**, **E**), and the effect was completely abolished by intrathecal pretreatment with AM630 (Fig. **1D**). These results indicated that $CB_2Rs$ in the spinal cord are the primary site of action for the suppressive effect of JWH133 administered systemically for treatment of PNI-induced tactile allodynia.

### Involvement of the $G_{i/o}$ Signaling Pathway in Suppressing Tactile Allodynia *via* $CB_2Rs$

We investigated the intracellular signaling pathway involved in $CB_2R$-mediated mitigation of tactile allodynia. Because $CB_2Rs$ are coupled with $G_{i/o}$-proteins, we examined the effect of the $G_{i/o}$-protein inhibitor PTx. Rats subjected to PNI were injected intrathecally with PTx (0.5 μg) and JWH133 (10 nmol). As shown in Fig. (**2A**), the ability of JWH133 to improve tactile allodynia was almost completely inhibited by PTx (Fig. **2A**). There was no change in PWT by

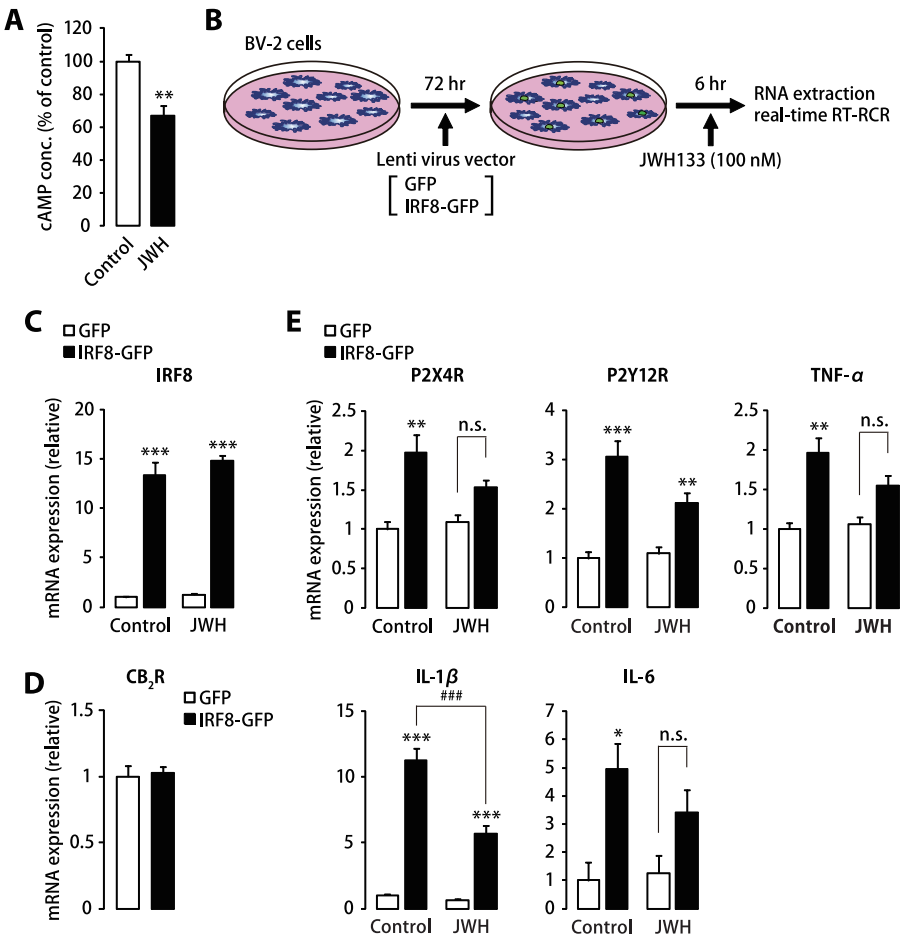


**Fig. (5).** The $CB_2R$ signal regulates the expression of pain-related molecules and suppresses inflammatory responses. (**A**) The concentration of cAMP was estimated 30 min after treatment with JWH133 (100 nM) on BV-2 cells (n=4; **p<0.01 *vs.* control). (**B**) Scheme of gene transfer experiments. Activated microglial cell cultures were prepared by overexpression of GFP-labeled IRF8 (IRF8-GFP) using lentiviral vectors. Changes in the expression of pain-related molecules were analyzed after JWH133 treatment. (**C**-**D**) After gene transduction with lentiviral vectors, the expression changes of IRF8 (**C**) and CB2R (**D**) mRNA were confirmed by real-time RT-PCR (n=4; ***p<0.001 *vs.* GFP). E. The expression changes of P2X4R, P2Y12R, TNF-α, IL-1β, IL-6, and IL-10 mRNA were analyzed by real-time RT-PCR after JWH133 (100 nM, 6h) treatment (n=4; ***p<0.001 *vs.* GFP; $^{###}$p<0.001 *vs.* control).

consistent with previous reports [18, 26]. A previous study in normal rats reported that activating $CB_2Rs$ in keratinocytes induced the release of β-endorphin and that an antinociceptive effect on noxious heat stimuli by the $CB_2R$ agonist AM1241 administered intraperitoneally was prevented by naloxone treatment in the hindpaw [17]. However, our study showed that intraplantar pretreatment with AM630 had no effect on the antiallodynic action of JWH133 given systemically in neuropathic rats. This suggested a minor role of $CB_2Rs$ in the skin in suppressing allodynia by JWH133 during neuropathic pain states. Interestingly, the level of $CB_2R$ expression in the spinal cord was very low under normal conditions [21], but it was upregulated after PNI, and was observed for at least 2 weeks after PNI. The change in $CB_2R$ expression in the spinal cord appears to parallel the behavioral effect of JWH133 in neuropathic rats because its effect on PWT was observed in the ipsilateral, but not the contralateral side. Furthermore, consistent with previous studies [21, 29], the expression of $CB_2R$ mRNA in the spinal cord was not increased following peripheral tissue inflammation caused by intraplantar injection of CFA. When $CB_2R$ agonists are administered intrathecally, they do not show profound antinociceptive effects in CFA-induced inflammatory pain [29]. Thus, it is assumed that upregulation of $CB_2R$ expression may be involved in producing an efficient cannabinoid action during neuropathic pain states and strongly supports our view that following PNI, the primary site of action of CB2R agonist is the spinal cord where they modulate agonist pain.

In addition to the acute reversal effect, we demonstrated that repeated intrathecal administration of JWH133 prevented the development of tactile allodynia after PNI. It seems likely that this preventive effect is not simply due to the residual acute antiallodynic effect of JWH133 because each behavioral measurement was performed when the effect of JWH133 on PWT was no longer observed (Fig. **1D**). However, when JWH133 was administered intrathecally from day 7 post-PNI, the $CB_2R$ agonist produced a slight increase in PWT. The reduction in JWH133 efficacy may be associated with the upregulation of $CB_2R$ expression during the maintenance phase. Together, our results suggest that $CB_2R$ agonists induce a potent reversal effect on established

tactile allodynia and prevent the development of tactile allodynia after PNI.

The ability to suppress PNI-induced allodynia by stimulating $CB_2Rs$ suggests that $CB_2Rs$ upregulated in the spinal cord may function as endogenous suppressors of neuropathic pain. However, intrathecal administration of $CB_2R$ antagonists did not exacerbate PNI-induced allodynia. It was also shown that inhibitors of fatty acid amide hydrolase and monoacylglycerol lipase enzymes, involved in the metabolism of endocannabinoids, attenuate neuropathic pain [30, 31]. Therefore, the levels of endocannabinoids that bind $CB_2Rs$ may not be sufficient to suppress PNI-induced neuropathic pain even though $CB_2R$ expression is upregulated. Thus, enhancing $CB_2R$ activity in the spinal cord by administering exogenous $CB_2R$ agonists might be an effective way to relieve neuropathic pain. As pharmacological stimulation of $CB_2Rs$ in the spinal cord did not affect the mechanical threshold in the contralateral side, a predicted therapeutic benefit of spinal $CB_2R$ activation is that normal pain sensitivity might be unaffected.

Several lines of evidence indicate that glial cells are the predominant cell type expressing $CB_2Rs$ in the spinal cord [21, 26, 27]. The time course of increased $CB_2R$ expression is similar to that of microglial activation, and the upregulation of $CB_2Rs$ also occurs in other diseases associated with microglial activation [32, 33]. It was reported that microglial activation in the spinal cord after PNI is suppressed by administration of $CB_2R$ agonists and in mice overexpressing $CB_2Rs$ [26, 34]. Furthermore, $CB_2R$ agonists decrease the level of the proinflammatory cytokine IL-1β in the spinal cord [27]. These findings suggest that glial cells, especially microglia, are the target cells involved in suppressing PNI-induced tactile allodynia by $CB_2R$ agonists. However, the underlying mechanisms remain to be fully determined. We recently demonstrated that the transcription factor IRF8 is a critical regulator for the reactive state of microglia after PNI and for neuropathic pain. In the present study, we demonstrated that JWH133 reduced the expression of IL-1β and also showed a tendency to decrease P2X4R, a purinergic receptor, and other proinflammatory cytokines (TNF-α and IL-6). P2X4R is a member of the ionotropic purinergic receptor [35] and is expressed in activated microglia after PNI [24] and other neurodegenerative diseases [36]. Importantly, P2X4Rs are essential for the pathogenesis of neuropathic pain [6, 24, 37]. Furthermore, IL-1β, IL-6, and TNF-α can enhance neuronal excitability in the spinal cord and contribute to neuropathic pain [1, 4, 38]. Although the present study did not examine the expression of these molecules at the protein level, it is speculated that activating spinal $CB_2Rs$ reduces the inflammatory responses derived from reactive microglia following PNI, which may be involved in suppressing the development of neuropathic pain. However, it seems unlikely that the acute reversal effect of JWH133 on established tactile allodynia is similarly explained by its effect on IRF8-induced gene expression because the antiallodynic effect of intrathecal administration of JWH133 was already observed 30 min after the injection. As the reversal of PNI-induced allodynia was cancelled by either the $G_{i/o}$ inhibitor PTx or AC activator forskolin, it implies the involvement of the $CB_2R$-$G_{i/o}$-AC signaling pathway. As there is increasing evidence that certain types of cannabinoid ligands also influence activity of T-type calcium channels at primary afferent sensory neurons *via* both $CB_2R$-dependent and independent manners [39-41], it is possible that these effects may be involved in the reversal of PNI-induced allodynia by JWH133. This possibility should be investigated in future studies.

## CONCLUSION

We demonstrated that $CB_2R$ in the spinal cord is upregulated after PNI and is the primary site of action for $CB_2R$ agonists administered systemically to improve established neuropathic pain through the $G_{i/o}$-AC signaling pathway. Activating spinal $CB_2Rs$ also suppressed the development of neuropathic pain. Furthermore, treatment with the $CB_2R$ agonist reduced the inflammatory response of microglia activated by overexpressing IRF8. Thus, spinal $CB_2R$ may be a therapeutic target to suppress the development and maintenance of neuropathic pain.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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## PATIENT'S CONSENT

Declared none.

## REFERENCES

[1] Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci 2009; 32: 1-32.

[2] Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000; 288: 1765-9.

[3] Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in "small" glia. Trends Neurosci 2005; 28: 101-7.

[4] McMahon SB, Malcangio M. Current challenges in glia-pain biology. Neuron 2009; 64: 46-54.

[5] Inoue K. The function of microglia through purinergic receptors: Neuropathic pain and cytokine release. Pharmacol Ther 2006; 109: 210-26.

[6] Coull JA, Beggs S, Boudreau D*, et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 2005; 438: 1017-21.

[7] Cravatt BF, Lichtman AH. The endogenous cannabinoid system and its role in nociceptive behavior. J Neurobiol 2004; 61: 149-60.

[8] Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. Br J Pharmacol 2008; 153: 319-34.

[9] Howlett AC, Barth F, Bonner TI*, et al.* International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 2002; 54: 161-202.

[10] Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006; 58: 389-462.

[11] Sharir H, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther 2010; 126: 301-13.

[12] Whiteside GT, Lee GP, Valenzano KJ. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of

small molecule CB2 receptor agonists. Curr Med Chem 2007; 14: 917-36.
[13] Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol 2010; 160: 467-79.
[14] Correa F, Mestre L, Docagne F*, et al.* Activation of cannabinoid CB2 receptor negatively regulates IL-12p40 production in murine macrophages: role of IL-10 and ERK1/2 kinase signaling. Br J Pharmacol 2005; 145: 441-8.
[15] Klegeris A, Bissonnette CJ, McGeer PL. Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. Br J Pharmacol 2003; 139: 775-86.
[16] Maresz K, Pryce G, Ponomarev ED*, et al.* Direct suppression of CNS autoimmune inflammation *via* the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. Nat Med 2007; 13: 492-7.
[17] Ibrahim MM, Deng H, Zvonok A*, et al.* Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci USA 2003; 100: 10529-33.
[18] Yamamoto W, Mikami T, Iwamura H. Involvement of central cannabinoid CB2 receptor in reducing mechanical allodynia in a mouse model of neuropathic pain. Eur J Pharmacol 2008; 583: 56-61.
[19] Ashton JC, Glass M. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. Curr Neuropharmacol 2007; 5: 73-80.
[20] Benito C, Tolon RM, Pazos MR*, et al.* Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol 2008; 153: 277-85.
[21] Zhang J, Hoffert C, Vu HK*, et al.* Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur J Neurosci 2003; 17: 2750-4.
[22] Masuda T, Tsuda M, Yoshinaga R*, et al.* IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. Cell Rep 2012; 1: 334-40.
[23] Chaplan SR, Bach FW, Pogrel JW*, et al.* Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53: 55-63.
[24] Tsuda M, Shigemoto-Mogami Y, Koizumi S*, et al.* P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 2003; 424: 778-83.
[25] Shiratori M, Tozaki-Saitoh H, Yoshitake M*, et al.* P2X7 receptor activation induces CXCL2 production in microglia through NFAT and PKC/MAPK pathways. J Neurochem 2010; 114: 810-9.
[26] Romero-Sandoval A, Nutile-McMenemy N, DeLeo JA. Spinal microglial and perivascular cell cannabinoid receptor type 2 activation reduces behavioral hypersensitivity without tolerance after peripheral nerve injury. Anesthesiology 2008; 108: 722-34.
[27] Luongo L, Palazzo E, Tambaro S*, et al.* 1-(2',4'-dichlorophenyl)-6-methyl-N-cyclohexylamine-1,4-dihydroindeno[1,2-c]pyraz ole-3-carboxamide, a novel CB2 agonist, alleviates neuropathic pain through functional microglial changes in mice. Neurobiol Dis 2010; 37: 177-85.
[28] Blasi E, Barluzzi R, Bocchini V*, et al.* Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. J Neuroimmunol 1990; 27: 229-37.
[29] Hsieh GC, Pai M, Chandran P*, et al.* Central and peripheral sites of action for CB(2) receptor mediated analgesic activity in chronic inflammatory and neuropathic pain models in rats. Br J Pharmacol 2011; 162: 428-40.
[30] Jhaveri MD, Richardson D, Kendall DA*, et al.* Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. J Neurosci 2006; 26: 13318-27.
[31] Desroches J, Guindon J, Lambert C*, et al.* Modulation of the anti-nociceptive effects of 2-arachidonoyl glycerol by peripherally administered FAAH and MGL inhibitors in a neuropathic pain model. Br J Pharmacol 2008; 155: 913-24.
[32] Benito C, Nunez E, Tolon RM*, et al.* Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. J Neurosci 2003; 23: 11136-41.
[33] Maresz K, Carrier EJ, Ponomarev ED*, et al.* Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem 2005; 95: 437-45.
[34] Racz I, Nadal X, Alferink J*, et al.* Interferon-gamma is a critical modulator of CB(2) cannabinoid receptor signaling during neuropathic pain. J Neurosci 2008; 28: 12136-45.
[35] Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol Rev 1998; 50: 413-92.
[36] Zhang Z, Zhang ZY, Fauser U*, et al.* Mechanical allodynia and spinal up-regulation of P2X4 receptor in experimental autoimmune neuritis rats. Neuroscience 2008; 152: 495-501.
[37] Beggs S, Trang T, Salter MW. P2X4R+ microglia drive neuropathic pain. Nat Neurosci 2012; 15: 1068-73.
[38] Kawasaki Y, Xu ZZ, Wang X*, et al.* Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med 2008; 14: 331-6.
[39] Chemin J, Monteil A, Perez-Reyes E*, et al.* Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. EMBO J 2001; 20: 7033-40.
[40] You H, Gadotti VM, Petrov RR*, et al.* Functional characterization and analgesic effects of mixed cannabinoid receptor/T-type channel ligands. Mol Pain 2011; 7: 89.
[41] Gadotti VM, You H, Petrov RR*, et al.* Analgesic effect of a mixed T-type channel inhibitor/CB2 receptor agonist. Mol Pain 2013; 9: 32.

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