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Activation of Cannabinoid CB_2 receptors Reduces Hyperalgesia in an Experimental Autoimmune Encephalomyelitis Mouse Model of Multiple Sclerosis

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Abstract

Clinical trials investigating the analgesic efficacy of cannabinoids in multiple sclerosis have yielded mixed results, possibly due to psychotropic side effects mediated by cannabinoid CB_1 receptors. We hypothesized that a CB_2-specific agonist (JWH-133) would decrease hyperalgesia in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. 4 weeks after induction of experimental autoimmune encephalomyelitis, we found that intrathecal administration of JWH-133 (10–100 μg) dose-dependently reduced both mechanical and cold hypersensitivity without producing signs of sedation or ataxia. The anti-hyperalgesic effects of JWH-133 could be dose-dependently prevented by intrathecal co-administration of the CB_2 antagonist, AM-630 (1–3 μg). Our results suggest that JWH-133 acts at CB_2 receptors, most likely within the dorsal horn of the spinal cord, to suppress the hypersensitivity associated with experimental autoimmune encephalomyelitis. These are the first pre-clinical studies to directly promote CB_2 as a promising target for the treatment of central pain in an animal model of multiple sclerosis.

Keywords

Pain; CB_2 receptor; JWH-133; AM-630; multiple sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune-inflammatory neurodegenerative disease of the central nervous system that afflicts well over 2 million people worldwide. MS disrupts the

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DISCLOSURE

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function of the brain, spinal cord and optic nerves and is characterized by acute inflammation, demyelination and axonal and neuronal loss. Neuropathic pain is one of the most frequent symptoms of MS, reported by roughly half of all patients [12]. MS patients present not only with spontaneous pain, but also several forms of evoked pain including cutaneous mechanical and cold hypersensitivity in the distal extremities [31, 39]. Despite its heavy impact on patient quality of life, pain in MS is often neglected or undertreated. Conventional analgesics either show low efficacy or produce unwanted side effects [29].

Emerging data suggest that activation of endogenous cannabinoid receptors can suppress neuropathic pain [23, 33]. Cannabinoid receptor (CB) subtypes include CB_1 and CB_2. CB_1 is predominately located in neurons within the central nervous system, while CB_2 is localized primarily on immune and microglial cells [28]. CB_2-immunoreactivity is significantly up-regulated at the lesion sites of post-mortem human spinal cord from patients with MS [47], as well as 4 weeks after $MOG_{33–55}$-induction of EAE in mice [24, 32].

Double-blind randomized controlled trials on the analgesic efficacy of the CB_1/CB_2 agonist Sativex, an oromucosal spray of tetrahydrocannabinol and cannabidiol, have yielded mixed results [18, 41]. Other CB agonist preparations such as delta-9-tetrahydrocannabinol or nabilone consistently reduce MS pain, but at the expense of dizziness, headache, fatigue and/or impaired judgment [38, 43, 48]. Mixed results are likely due in part to the psychotropic side effects associated with CB_1 activation, and point to the CB_2 as an attractive target for chronic pain [2, 25]. To date, however, an exhaustive literature search indicates that no study has evaluated the efficacy of a CB_2 selective agonist for pain in MS. Here we address this question in a validated experimental autoimmune encephalomyelitis (EAE) mouse model. EAE is associated with neurodegenerative pathology, and a behavioral phenotype that is reflective of MS in humans, including the development of cutaneous mechanical and cold hypersensitivity [30, 34]. We hypothesized that intrathecal administration of a CB_2-selective agonist, JWH-133 [17, 46], would decrease mechanical and cold hypersensitivity without locomotor side effects, and that this would be blocked by the CB_2-selective antagonist, AM-630.

Materials and Methods

EAE Model

Our EAE model was designed with reagent concentrations that produce a mild-to-moderate form of EAE, always yielding clinical scores of motor dysfunction below 3 and 4 over the 4 week testing period [34]. All animals in the current study exhibited neither paresis of both hindlimbs nor paralysis of one hindlimb, thus allowing nociceptive paw reflex testing. Briefly, myelin oligodendrocyte glycoprotein 35–55 ($MOG_{33–55}$, AnaSpec Inc, Fremont, CA) was emulsified in a 1:1 solution of 1x PBS and complete Freund's adjuvant (CFA). To minimize the hyperalgesia produced by CFA itself, we reduced its concentration to 3 mg/ml. We used female C57BL/6 mice (Charles River), aged 12–14 weeks, housed 4 to a cage, habituated for 1 week in a temperature and humidity controlled environment on a 14/10-h light/dark cycle. Food and water were available *ad libitum*. Procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky, protocol (#2010-0770). MOG was bilaterally injected (150 μg/100 μl s.c.) at the flanks under light

isoflurane anesthesia on Days 0 and 6. Pertussis toxin (List Biological Laboratories, Campbell, CA) was injected (200 ng/200 μl, i.p.) on days 0 and 2 to facilitate opening of the blood brain barrier to T cells by histamine-induced vascular leakage and/or the priming of autoreactive T cells [16]. Control mice received CFA and pertussis toxin, but not MOG. Average weight of mice on week 4 was 21.8 ± 0.2 g.

Intrathecal (i.t.) Drug Administration

Intrathecal injection was performed in lightly restrained unanesthetized mice as previously described [11]. We injected 5 μl of drug [JWH-133 (Tocris, UK), AM-630 (Tocris, UK), or WIN55,212-2 (Cayman Chemical, Ann Arbor, MI) and/or vehicle [ethanol: alkamuls EL-620 (Rhodia, Cranbury, NJ): saline in a volume ratio of 1:1:8]. The data of Figure 1 includes animals that were injected twice (Days 24 and 28 after the first MOG injection) using a balanced crossover design. Animals receiving vehicle on day 24 received drug on day 28, and vice versa. Group means of either vehicle or drug on day 24 and day 28 did not differ, and so were combined for final analysis. The data of Figure 2 were obtained on day 28 after two consecutive i.t. injections, separated by 15 min: first AM-630 or vehicle and then JWH-133, followed by behavioral testing sessions.

Behavioral Testing

All animals were acclimated to a stainless steel grid within individual Plexiglas tubes for 30 to 60 min prior to behavioral testing. Somatosensory testing using mechanical (von Frey hair) and cold (plantar application of 5–8 μL acetone) stimuli was conducted as previously described [37]. All experiments were conducted by a female investigator (W.F.), blinded to model and identity of drugs.

Statistics

In Figs. 1A–B and 2A–B, differences between means were analyzed by two-way analysis of variance (ANOVA). Drug/Dose was a grouping factor and time was the repeated measure. If a significant interaction was found ($p < 0.05$), ANOVA was followed by post-hoc Bonferroni tests. Data were re-plotted as area under the curve, calculated using the trapezoidal method (Figs. 1C–D and 2C–D). Effects of Drug were analyzed by one-way ANOVA followed by post-hoc Dunnett multiple comparison test. The best fit line for dose-response curves (Figs. 1E–F) was generated following non-linear regression analysis based on the 60 min post-injection thresholds. % Maximum Possible Effect (MPE) was calculated as:

$$MPE = \text{postinjury threshold} - \text{preinjection threshold}$$
$$\%MPE = \frac{\text{postinjection threshold} - \text{preinjection threshold}}{\text{MPE}} \times 100$$

All data are presented as mean ± SEM. ★$p < 0.05$ was considered statistically significant.

Results

CB_2 agonist JWH-133 reduced EAE hypersensitivity in a dose-dependent manner

We first tested the hypothesis that activation of spinal CB_2 suppresses mechanical and cold hypersensitivity in EAE mice. As illustrated in Figs 1A–D, at doses based on previous analgesia studies in mice [6, 21, 46], JWH-133 dose-dependently reduced mechanical (1A, $F_{4, 33} = 32.5$, $p < 0.0001$) and cold hypersensitivity (1B, $F_{4, 33} = 2.8$, $p < 0.05$). The anti-hyperalgesic effect of JWH-133 (100 μg) for mechanical and cold hypersensitivity peaked at 60 and 30 min respectively, with restoration of thresholds to baseline levels (0.77 ± 0.08 g, $p > 0.05$ vs baseline; 2.52 ± 0.05 s, $p > 0.05$ vs baseline, respectively) within 180 min. Area under the curve (AUC) analysis (0–180 min) illustrates the concentration-dependent actions of JWH-133 (1C–D, $p < 0.01$). As illustrated in Figs. 1E–F, dose-response curves yielded EC_{50} values of 49.0 μg and 33.5 μg for the mechanical and cold modalities, respectively. As illustrated in Supplementary Figure S1, JWH-133 (100 μg) did not change rotarod latency ($p > 0.05$).

CB2 antagonist AM-630 prevented the anti-hyperalgesic effects of JWH-133

To further evaluate CB_2 as the target of JWH-133, we intrathecally administrated 100 μg JWH-133 followed by the highly selective CB_2 antagonist AM-630 [35] at intrathecal doses in the low μg range [13, 19]. We did not include an AM-630 alone control group because these doses do not change sensory thresholds [9, 19]. AUC analysis illustrates that AM-630 dose-dependently attenuated the inhibitory effects of 100 μg JWH-133 on mechanical (2C, $F_{2, 11} = 15.0$, $p < 0.001$) and cold hypersensitivity (2D, $F_{2, 11} = 4.2$, $p < 0.05$).

Discussion

CB_2 is an emerging target for pain relief as suggested by clinical trials and data from animal models of chronic pain [2, 5, 33]. For example, CB_2 mRNA or protein levels were up-regulated in the spinal cord after peripheral nerve injury in rat [42, 44, 49]. Furthermore, intrathecal administration of CB_2 selective agonists generally reduced hyperalgesia in rodent models of peripheral neuropathic pain (as reviewed in [33], but see [4]). These anti-hyperalgesic effects were abolished in CB_2 knockout animals [46] or by co-administration of a CB_2 selective antagonist [3, 13, 25], advancing a spinal CB_2 site of activation. The current results extend these findings to the EAE model of multiple sclerosis pain. We found that the CB_2 agonist JWH-133 reduced mechanical and cold hypersensitivity in EAE mice in a dose-dependent manner. CB_2 deletion mutant mice exhibit more severe disease scores [26], while chronic systemic administration of CB_2 agonists ameliorated disease progression in EAE animals [22, 32]; however, it is highly unlikely that a single injection of the CB_2 agonist JWH-133 could impact disease progression within the 3 hr window of behavioral observation in the current study. Pre-treatment with the CB_2 antagonist, AM-630, reversed the anti-hyperalgesic effects of JWH-133, suggesting a contribution of spinal CB_2 to pain control in the EAE model. Consistent with this conclusion, $MOG_{35–55}$ increased CB_2 mRNA and protein levels in the spinal cord of EAE animals [24, 32], and CB_2-immunoreactivity is significantly up-regulated at the lesion sites of postmortem human spinal cord from patients with MS [47]. Our results are consistent with the analgesic effects

of oral cannabinoid-based medications in patients with spinal cord injury [14, 27] and of CB_2 agonists in rodents with spinal cord injury [1, 15].

Previous studies in a mouse model of nerve injury reported that intrathecal administration of 31 μg of JWH-133 (approximately 1.5–2.0 mg/kg) reduced behavioral signs of peripheral neuropathic pain, suggesting a spinal site of action [46]. Similarly, in the current study we found that intrathecal doses of up to 100 μg of JWH-133 reduced behavioral signs of central neuropathic pain. Although we did not attempt injection of an equivalent systemic dose of JWH-133 (4.5 mg/kg, based on our average mouse weight of 22g), previous studies in the CFA model of inflammatory pain or the brachial plexus avulsion model of peripheral neuropathic pain indicate that systemic doses at or exceeding 10 mg/kg for JWH-133 are required to exert anti-hyperalgesic effects [7, 8]. Similarly, a 5 mg/kg i.p. dose of JWH-133 failed to reduce pain thresholds in the second phase of the formalin model of ongoing inflammatory pain [20]. These data support our conclusion that intrathecal injection of JWH-133 acts at spinal sites and does not diffuse out of the spinal cord at sufficient concentrations to exert its anti-hyperalgesic actions at peripheral CB_2. Further supporting a spinal site of action, we found that intrathecal injection of a low dose (3 μg) of the CB_2 antagonist AM-630 blocked the anti-hyperalgesic effects of intrathecal JWH-133. This low dose is equivalent to 0.135 mg/kg (based on our average mouse weight of 22g), which is much lower than the 3mg/kg i.p. dose of AM-630 that was used to block the effect of 10mg/kg JWH-133 [7, 8], and so is unlikely to produce systemic effects. However, we cannot entirely exclude a contribution of peripheral CB_2 at the intrathecal doses of JWH-133 used in our study because JWH-133 (1–10 mg/kg i.p.) reduced weight bearing in rat models of cisplatin-induced neuropathic pain and carrageenan-induced inflammatory pain [10, 40].

Our rotarod data indicate that WIN55-212,2 produces ataxia, consistent with previous reports in rodents [25], and a consensus that activation of central CB_1 causes psychotropic effects and therefore compromises the therapeutic efficacy of cannabinoids to reduce clinical chronic pain, including MS pain [33, 36]. By contrast, we found that JWH-133 reduced hypersensitivity at doses that do not produce ataxia. This is consistent with previous studies in other pain models indicating an absence of motor impairment or catalepsy following systemic injection of analgesic 20 mg/kg doses JWH-133 [45]. Although JWH-133 exhibits substantial selectivity (200 X) for CB2 over CB1 [17], we cannot exclude a contribution of CB1 receptors located in the DRG and spinal cord to its analgesic actions because we did not administer it in combination with a CB1 antagonist. Thus, our data suggest that selective targeting of spinal CB_2 reduces behavioral signs of neuropathic pain in the EAE model without untoward side effects. Taken together with an emerging literature indicating that inhibitory effects of CB_2 activation on the immune system may reduce the progression of neuromuscular dysfunction in the EAE model [22, 32], we conclude that the targeting of central CB_2 poses strong therapeutic potential for the treatment of both motor dysfunction and pain in MS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- CB_2 receptor activation with JWH-133 reduces central neuropathic pain in mice.
- The site of anti-hyperalgesic action of JWH-133 includes the spinal cord.
- JWH-133 reduces hypersensitivity at doses that do not produce ataxia.
- First pre-clinical studies to promote CB_2 for treatment of multiple sclerosis pain.

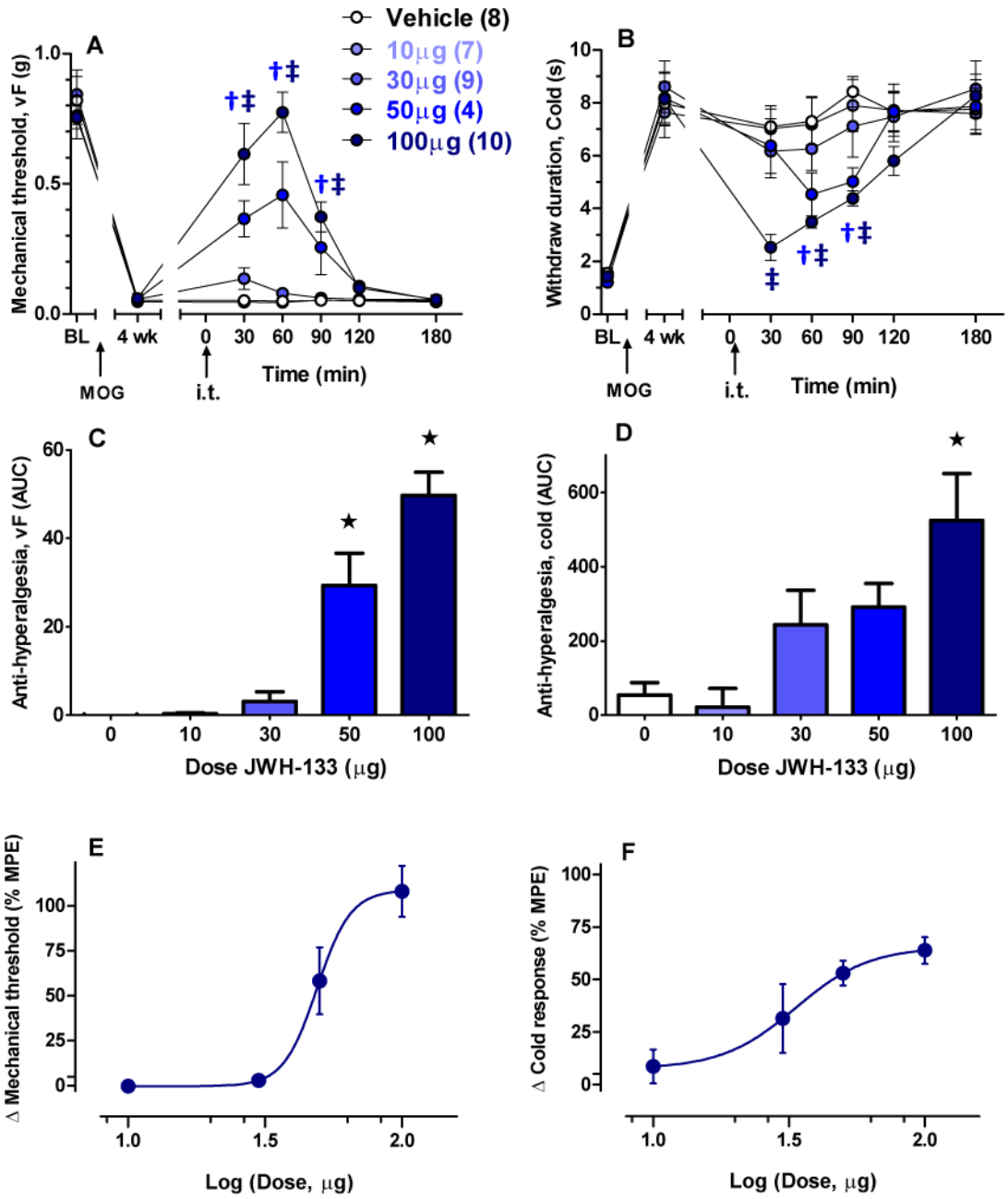

Figure 1. Intrathecal JWH-133 suppresses mechanical and cold hypersensitivity in a dose-dependent manner

The CB_2-selective agonist JWH-133 was intrathecally (i.t.) administered 24–28 d (4 wk) after induction of EAE in C57/B6 mice. Time course and area under the curve (AUC) analysis of mechanical (**A,C**) and cold (**B,D**) hypersensitivity after JWH-133. Dose-response analysis of the data at 60 min for mechanical (**E**) and cold hypersensitivity (**F**), respectively. MPE: maximum possible effect. Parentheses refer to number of animals per group. $^{\dagger}p < 0.05$ 50 μg vs vehicle; $^{\ddagger}p < 0.05$ 100 μg vs vehicle; $^{\star}p < 0.05$ vs vehicle.

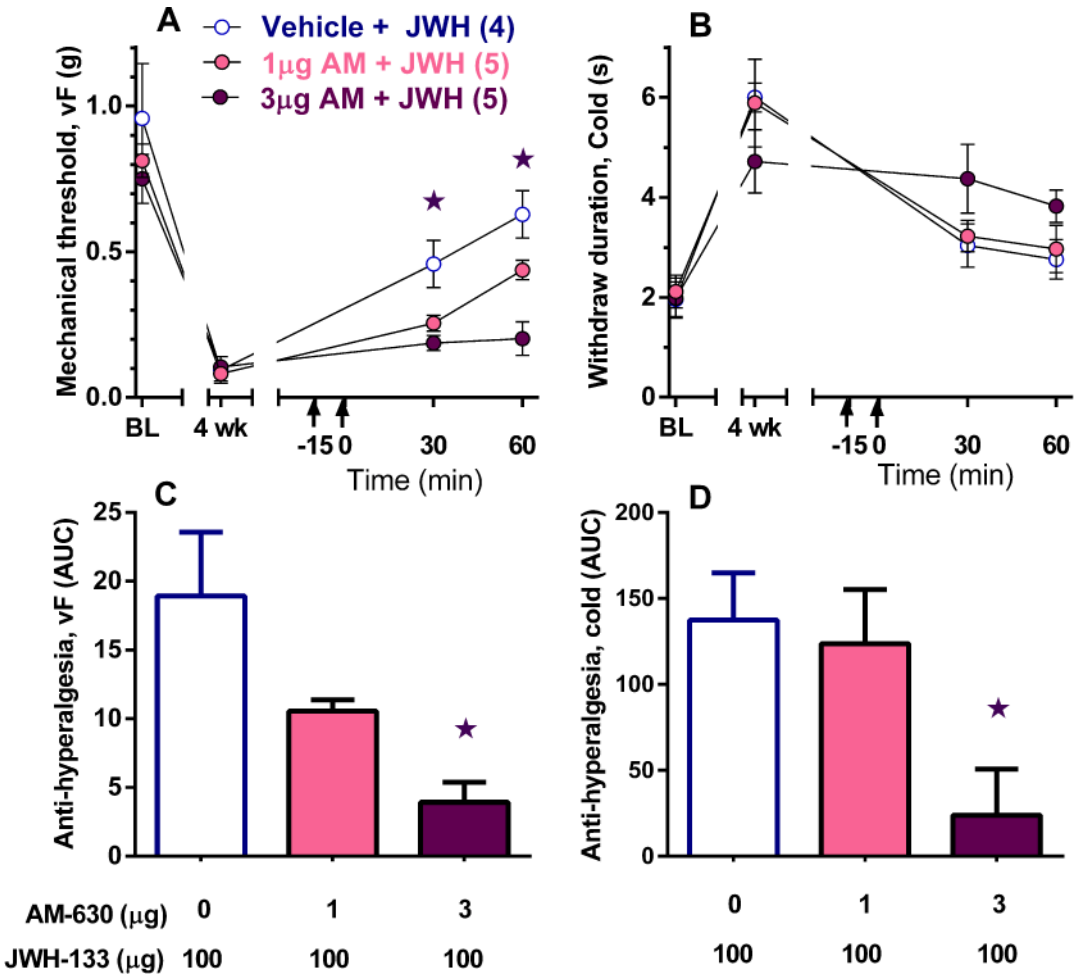

Figure 2. CB_2 antagonist blocks the anti-hyperalgesic effects of JWH-133

The CB_2 antagonist AM-630 was i.t. administered 15 min prior to i.t. JWH-133 (100 μg). Time course and AUC analysis (0–60 min) indicate that AM-630 attenuated the anti-hyperalgesic effects of JWH-133 on mechanical (**A, C**) and cold (**B, D**) responses. ★different from vehicle. ★$p < 0.05$ vs vehicle.