CB₁ Knockout Mice Unveil Sustained CB₂-Mediated Antiallodynic Effects of the Mixed CB₁/CB₂ Agonist CP55,940 in a Mouse Model of Paclitaxel-Induced Neuropathic Pain

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ABSTRACT

Cannabinoids suppress neuropathic pain through activation of cannabinoid CB₁ and/or CB₂ receptors; however, unwanted CB₁-mediated cannabimimetic effects limit clinical use. We asked whether CP55,940 ([-3]-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexanol), a potent cannabinoid that binds with similar affinity to CB₁ and CB₂ in vitro, produces functionally separable CB₁- and CB₂-mediated pharmacological effects in vivo. We evaluated antiallodynic effects, possible tolerance, and cannabimimetic effects (e.g., hypothermia, catalepsy, CB₁-dependent withdrawal signs) after systemic CP55,940 treatment in a mouse model of toxic neuropathy produced by a chemotherapeutic agent, paclitaxel. The contribution of CB₁ and CB₂ receptors to in vivo actions of CP55,940 was evaluated using CB₁ knockout (KO), CB₂KO, and wild-type (WT) mice. Low-dose CP55,940 (0.3 mg/kg daily, i.p.) suppressed paclitaxel-induced allodynia in WT and CB₂KO mice, but not CB₁KO mice. Low-dose CP55,940 also produced hypothermia and rimonabant-precipitated withdrawal in WT, but not CB₂KO mice. In WT mice, tolerance developed to CB₁-mediated hypothermic effects of CP55,940 earlier than to antiallodynic effects. High-dose CP55,940 (10 mg/kg daily, i.p.) produced catalepsy in WT mice, which precluded determination of antiallodynic efficacy but produced sustained CB₂-mediated suppression of paclitaxel-induced allodynia in CB₂KO mice; these antiallodynic effects were blocked by the CB₂ antagonist 6-iodopravadoline (AM630). High-dose CP55,940 did not produce hypothermia or rimonabant-precipitated withdrawal in CB₂KO mice. Our results using the mixed CB₁/CB₂ agonist CP55,940 document that CB₁ and CB₂ receptor activations produce mechanistically distinct suppression of neuropathic pain. Our study highlights the therapeutic potential of targeting cannabinoid CB₂ receptors to bypass unwanted central effects associated with CB₁ receptor activation.

Introduction

Peripheral neuropathic pain induced by chemotherapy detrimentally impacts the lives of cancer patients and is one of the major side effects responsible for discontinuation of anticaner treatment (Windebank and Grisold, 2008). To date, the mechanisms underlying chemotherapy-induced neuropathic pain remain poorly understood (Windebank and Grisold, 2008). Additional challenges for the management of chemotherapy-induced neuropathy include limited efficacy and significant side effects of existing medications (Pachman et al., 2011). Thus, identification of therapeutic strategies that are both safe and effective for managing chemotherapy-evoked neuropathic pain remains an unmet clinical need.

Cannabinoids produce antinoceptive effects in preclinical models of neuropathic pain (Herzberg et al., 1997; Ibrahim et al., 2003; Guindon and Hohmann, 2009; Kinsey et al., 2009; Pertwee, 2009; Schlosburg et al., 2009); however, unwanted psychotropic effects of cannabinoids limit their potential clinical use (Ben Amar, 2006; Pertwee, 2009; Dhopolshwarkar and Mackie, 2014). Two major subtypes of cannabinoid receptors, CB₁ and CB₂, are the key receptors responsible for the pharmacological effects of cannabinoids (Mackie, 2006). Both CB₁ and CB₂ receptors are G-protein-coupled receptors whose signaling pathways include inhibition of adenylyl cyclase and activation of mitogen-activated protein kinases (Mackie, 2006). CB₁ receptors are predominantly located in the central nervous system (CNS), whereas CB₂ receptors are found primarily in immune cells (Galiegue et al., 1995; Schatz et al., 1997; Marsicano and Lutz, 1999; Onaivi et al., 2006) and are upregulated in the CNS in response to inflammation or injury (Zhang et al., 2003; Maresz et al., 2005). Evaluation of the receptor mechanisms underlying therapeutic and psychotropic effects of cannabinoids, after both acute and chronic administration, may facilitate the development of safe and effective cannabinoid-based pharmacotherapies (Ben Amar, 2006; Pertwee, 2009).

CP55,940 ([-3]-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexanol) is a potent nonselective...
CB2 (Howlett et al., 2002; Atwood et al., 2012). Thus, cannabinoid CB1 and CB2 receptor subtypes to the in vivo, particularly in persistent pain states in which chronic CB2-mediated antinociceptive effects, rather than being abated by CB1 but not by CB2 receptors (Sain et al., 2009). This mice reported that the antinociceptive effects of systemic agonist that has not shown functional selectivity at CB1 or ligand-biased selectivity for different signal transduction with the dose that is sufficient to activate CB1) and that of CP55,940 has been reported to possess antinociceptive efficacy in various preclinical pain models, including acute pain, inflammatory pain, and neuropathic pain induced by traumatic nerve injury (Lichtman and Martin, 1997; Holmman et al., 1999; Romero et al., 2002; Scott et al., 2004; Choong et al., 2007; Sain et al., 2009). Pharmacological antagonism of CB1 receptors alone (Lichtman and Martin, 1997, Romero et al., 2002; Choong et al., 2007) or of both CB1 and CB2 receptors (Scott et al., 2004) blocks the antinociceptive effects of CP55,940 in rats; however, a study using CB1KO and CB2KO mice reported that the antinociceptive effects of systemic CP55,940 (at 0.3 mg/kg, i.p.), administered acutely, is mediated by CB1 but not by CB2 receptors (Sain et al., 2009). This finding led to the conclusion that agonist activity at CB2 is not relevant to antinociceptive effects of mixed CB1/CB2 agonists, at least following systemic administration. By contrast, we hypothesized that, due to the abundance of CB1 receptors (relative to CB2 receptors) in the CNS, higher doses of mixed cannabinoids are required to activate CB2 receptors (compared with the dose that is sufficient to activate CB1) and that CB2-mediated antinociceptive effects, rather than being absent, are masked by CB1-mediated catatonia associated with mixed cannabinoid agonists. The differences between the in vitro and in vivo profiles of CP55,940 raise questions about the differential roles and functions of CB1 and CB2 receptors in vivo, particularly in persistent pain states in which chronic dosing is required for clinical use.

In the present study, we investigated the contribution of cannabinoid CB1 and CB2 receptor subtypes to the in vivo actions of the mixed cannabinoid CP55,940 in a mouse model of chemotherapy-induced neuropathy using transgenic (i.e., CB1KO, CB2KO) and wild-type (WT) mice. We evaluated antinociceptive effects, possible tolerance, and typical CNS-associated side effects (e.g., hypothermia, catalepsy, and physical withdrawal) associated with chronic administration of CP55,940 at multiple doses. CB1KO mice were used to test the hypothesis that CB2-mediated signaling can be engaged by mixed cannabinoids in vivo to produce sustained antiallodynic efficacy without producing side effects. Thus, under conditions in which confounding effects of CP55,940 at CB1 receptors are absent (e.g., in CB1KO mice), CB2-mediated effects can be fully characterized to ascertain the therapeutic potential of targeting CB2 receptors.

Materials and Methods

Subjects. Adult CB1KO and WT littermates on a CD1 background and adult CB2KO and WT littermates on a C57BL/6J background weighing 26–35 g and of both sexes, were used in these experiments. CB1KO (B6.129P2-CNR1J) and CB2KO (B6.129P2-CNR2tm1DgenJ) and corresponding WT (C57BL/6J) mice were originally purchased from Jackson Laboratory (Bar Harbor, ME). CB1KO mice were generated as previously described (Ledent et al., 1999), whereas corresponding WT litters were periodically outcrossed with CD1 mice (strain number 022) from Charles River Laboratories (Wilmington, MA) to maintain genetic diversity. Animals were single housed in a temperature-controlled facility (73 ± 2°F, 45% humidity, regular 12-hour light/dark cycle, lights on at 7 AM), with food and water ad libitum. All experimental procedures were approved by the Bloomington Institutional Animal Care and Use Committee of Indiana University and followed the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

Drugs and Chemicals. Paclitaxel was purchased from TeClon Corporation (Irvine, CA) and was dissolved in cremophor-vehicle (1:1:18 ratio of cremophor/ethanol/saline). Cremophor EL, ethanol, dimethyl sulfoxide, and acetone were purchased from Sigma-Aldrich (St. Louis, MO). Alkamuls EL-620 was obtained from Rhodia (Cranbury, NJ). Saline was purchased from Aqualite System (Hospira, Lake Forest, IL). (-)CP55,940 (CP55,940) (Compton et al., 1992) was provided by the National Institute on Drug Abuse or purchased from Santa Cruz Biotechnology (Dallas, TX). Rimonabant (Yoshioka et al., 1989) was provided by the National Institute on Drug Abuse. AM630 (6-iodopraavadoline) (Ross et al., 1999) was purchased from Cayman Chemical Company (Ann Arbor, MI). CP55,940, rimonabant, and AM630 were dissolved in vehicle (5:2:2:16 ratio of dimethylsulfoxide/alkamuls –CP55,940 (CP55,940) (Compton et al., 1992) was provided by the International Association for the Study of Pain (Zimmermann, 1983).
In experiment 5, we examined the receptor mechanism underlying the antiallodynic effects of CP55,940 (3 mg/kg daily, i.p. × 8 days) in CB1KO mice. Paclitaxel-treated CB1KO mice that received vehicle, CP55,940 (3 mg/kg daily, i.p. × 8 days) alone or coadministered with AM630 (5 mg/kg daily, i.p. × 8 days) were examined.

**Assessment of Mechanical Alloodynia.** Withdrawal thresholds (in grams) to mechanical stimulation were measured in duplicate for each paw using an electronic von Frey anesthesiometer supplied with a 90-g range probe (ITC Life Science Inc., Woodland Hills, CA) as described previously (Deng et al., 2012, 2015). Briefly, mice were individually placed in transparent plastic chambers on an elevated metal mesh table and habituated to the testing apparatus for 30 minutes before testing. When animals ceased exploratory behaviors, a force was applied to the midplantar region of the hind paw by a semiflexible tip connected to the anesthesiometer. Mechanical stimulation was terminated on paw withdrawal.

**Assessment of Cold Alloodynia.** Response time (in seconds) to cold stimulation was assessed in triplicate for each paw by the acetone method as described previously (Deng et al., 2015). Briefly, mice were individually placed underneath transparent plastic chambers on an elevated metal mesh table. After habituation, an acetone bubble that formed at the end of a blunt 1-ml syringe was gently presented onto the plantar surface of the hind paw. Time that the animal spent motionless on the ring during a 5-minute observation period was recorded.

**Evaluation of Cannabinoid Withdrawal Symptoms.** WT mice were treated chronically with vehicle or CP55,940 (0.3 mg/kg per day, i.p.) for 9 days. CB2KO mice were treated chronically with vehicle or CP55,940 (0.3 or 3 mg/kg daily, i.p.) for 30 days before testing. When animals ceased exploratory behaviors, a force was applied to the midplantar region of the hind paw by a semiflexible tip connected to the anesthesiometer. Mechanical stimulation was terminated on paw withdrawal.

**Rectal Temperature.** Rectal temperature (°C) was measured using a thermometer (Physitemp Instruments Inc., Clifton, NJ) equipped with a thermometer (Physitemp Instruments Inc., Clifton, NJ) equipped with a thermometer (Physitemp Instruments Inc., Clifton, NJ) equipped with an electronic von Frey anesthesiometer supplied with a 90-g range probe (ITC Life Science Inc., Woodland Hills, CA) as described previously. Briefly, mice were individually placed in transparent plastic chambers on an elevated metal mesh table. After habituation, an acetone bubble that formed at the end of a blunt 1-ml syringe was gently presented onto the plantar surface of the hind paw. Time that the animal spent motionless on the ring during a 5-minute observation period was recorded.

**Ring Test.** Catalepsy was assessed using the ring test as previously described (Pertwee, 1972). Immobility latency (in seconds) that the animal spent motionless on the ring during a 5-minute observation period was recorded.

**Statistical Analyses.** Analysis of variance for repeated measures was used to determine the time course of allodynia and chronic drug effects. The sphericity-assumed correction was applied to all repeated measures, as described previously. 

**Results**

**Paclitaxel-Induced Alloodynia Developed Similarly in WT, CB1KO, and CB2KO Mice.** Before paclitaxel treatment, there were no genotype or gender differences in responses to mechanical (CB1: \( P = 0.70 \), Fig. 1A; CB2: \( P = 0.22 \), Fig. 1C) or cold stimulation (CB1: \( P = 0.15 \), Fig. 1B; CB2: \( P = 0.89 \), Fig. 1D).

Paclitaxel decreased thresholds to mechanical stimulation \((F_{3,62} = 72.33, P < 0.0001; \) Fig. 1A) and increased response time to cold stimulation \((F_{3,62} = 19.04, P < 0.0001; \) Fig. 1B) in CB2KO and WT littermates. Similarly, paclitaxel induced hypoallodynia \((P_{3,23} = 14.02, P < 0.0001; \) Fig. 1C) and cold \((F_{3,88} = 71.71, P < 0.0001; \) Fig. 1D) stimulation in both CB2KO and WT mice. Mechanical and cold allodynia were established in mice treated with paclitaxel relative to CR-vehicle group beginning on day 4 and were sustained throughout the testing interval in CB2KO, CB2KO, and WT mice \((P < 0.0001)\). Paclitaxel-induced mechanical and cold allodynia developed equivalently in CB2KO and WT mice, as well as in CB2KO and WT mice across all time points \((P = 1.00)\), consistent with our previously published work (Deng et al., 2015).

**Chronic Low-Dose CP55,940 Suppressed Paclitaxel-Induced Alloodynia in WT but Not in CB2KO Mice.** In WT mice, chronic low-dose treatment with CP55,940 (0.3 mg/kg daily, i.p.) reversed paclitaxel-induced mechanical \((F_{1,9} = 91.73, P < 0.0001; \) Fig. 2A) and cold \((F_{1,9} = 26.84, P < 0.002; \) Fig. 2B) alloodynia relative to vehicle \((P < 0.0001 for all comparisons)\) and preinjection levels \((F_{1,36} = 18.58, P < 0.0001 mechanical; F_{1,36} = 17.11, P < 0.0001 cold)\). The antiallodynic effects of low-dose CP55,940 were stable from treatment day 1 to day 8 \((F_{2,18} = 0.42, P = 0.66 mechanical; F_{2,18} = 1.50, P = 0.25 cold)\). Low-dose CP55,940 fully suppressed paclitaxel-induced allodynia and restored responses to prepaclitaxel baseline levels \((F_{3,2} = 2.24, P = 0.12 mechanical; F_{3,20} = 0.93, P = 0.44 cold; \) Fig. 2, A and B) in WT mice.

By contrast, in CB2KO mice, CP55,940 (0.3 mg/kg daily, i.p.) failed to attenuate paclitaxel-evoked mechanical \((F_{1,8} = 0.31, P = 0.60; \) Fig. 2C) or cold hypersensitivities \((F_{1,8} = 1.79, P = 0.22; \) Fig. 2D) relative to vehicle \((P > 0.07 for all comparisons)\) on any day \((F_{4,33} = 0.93, P = 0.46 mechanical; F_{4,33} = 0.98, P = 0.43 cold)\).

**Chronic Low-Dose CP55,940 Attenuated Paclitaxel-Induced Alloodynia in CB2KO Mice.** Chronic low-dose treatment with CP55,940 (0.3 mg/kg daily, i.p.) suppressed paclitaxel-induced mechanical \((F_{2,21} = 88.71, P < 0.0001; \) Fig. 2E) and cold \((F_{2,21} = 62.59, P < 0.0001; \) Fig. 2F) alloodynia relative to vehicle \((P < 0.0001 for all comparisons)\) and preinjection levels \((F_{2,20} = 30.20, P < 0.0001 mechanical; F_{12,84} = 20.81, P < 0.0001 cold)\) in both CB2KO and WT mice. CP55,940-induced antiallodynic effects were stable from treatment day 1 to 8 \((F_{4,42} = 0.67, P = 0.67 mechanical; F_{4,42} = 0.46, P = 0.83 cold)\) and normalized responses to prepaclitaxel baselines, followed by Bonferroni post hoc tests or two-tailed \( t \) tests, as appropriate. No gender differences were detected in chemotherapy-induced responses to mechanical or cold stimulation \((P > 0.41 for all comparisons)\) or in the effects of CP55,940 \((P > 0.15 for all comparisons)\); therefore, results from both genders were pooled for statistical analyses. Statistical analyses were performed using IBM-SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL). \( P < 0.05 \) was considered significant.
Chronic Low-Dose CP55,940 Produced Transient Hypothermia in Paclitaxel-Treated WT but Not CB1KO Mice. Chronic low-dose administration of CP55,940 (0.3 mg/kg daily, i.p.) decreased body temperature relative to vehicle on treatment day 2 ($P < 0.0001$), but not day 7 ($P = 0.20$), in paclitaxel-treated WT mice (Fig. 4A). Thus, tolerance developed to the hypothermic effect of low-dose CP55,940 after repeated dosing. By contrast, the same dosing paradigm failed to alter body temperature in paclitaxel-treated CB1KO mice on any day tested ($P = 0.07$; Fig. 4B), consistent with classic CB1-mediated hypothermia.

Chronic Low-Dose CP55,940 Produced Cannabinoid Withdrawal Signs in Paclitaxel-Treated WT but Not CB1KO Mice. In paclitaxel-treated WT mice, rimonabant (10 mg/kg, i.p.) challenge produced prototypical CB1-mediated withdrawal signs, such as paw tremors ($F_{3,20} = 57.79$, $P < 0.0001$) and headshakes ($F_{3,20} = 8.59$, $P < 0.002$) in mice treated chronically with low-dose CP55,940 (0.3 mg/kg daily, i.p.) relative to vehicle challenge ($P < 0.01$) and relative to the vehicle group ($P < 0.01$; Fig. 4B). Challenge with rimonabant, but not vehicle, elicited scratching behaviors in paclitaxel-treated WT animals that received chronic administration of vehicle or CP55,940 ($F_{3,20} = 7.88$, $P < 0.002$); WT mice treated with chronic CP55,940 (0.3 mg/kg daily, i.p.) showed more rimonabant-produced scratching behaviors compared with the vehicle group ($P < 0.05$; Fig. 5A).

By contrast, rimonabant challenge did not elicit paw tremors ($F_{3,12} = 0.62$, $P = 0.62$), headshakes ($F_{3,12} = 1.80$, $P = 0.20$), or scratching bouts ($F_{3,12} = 1.34$, $P = 0.31$) relative to vehicle challenge in CB1KO mice receiving either chronic CP55,940 (0.3 mg/kg daily, i.p.) or chronic vehicle (Fig. 5B), suggesting that rimonabant-induced scratching in the vehicle-treated mice is mediated by CB1 receptors.

Acute High-Dose CP55,940 Produced Catalepsy and Severe Hypothermia in WT but Not in CB1KO Mice. In paclitaxel-treated WT mice, a higher dose (3 mg/kg, i.p.) of CP55,940, administered acutely, induced catalepsy ($F_{2,14} = 391.89$, $P < 0.0001$; Fig. 6A) and hypothermia ($F_{2,14} = 35.59$, $P < 0.0001$; Fig. 6C) relative to either vehicle ($P < 0.0001$) or low-dose CP55,940 (0.3 mg/kg, i.p.) treatment ($P < 0.02$). By contrast, in paclitaxel-treated CB1KO mice, neither doses of CP55,940 (0.3 or 3 mg/kg, i.p.) altered immobility latency ($F_{2,12} = 0.75$, $P = 0.50$; Fig. 6B) or body temperature ($F_{2,12} = 1.19$, $P = 0.34$; Fig. 6D) relative to vehicle treatment.
Acute CP55,940 Dose-Dependently Suppressed Paclitaxel-Induced Neuropathy in CB1KO Mice. In CB1KO mice, acute systemic treatment with CP55,940 suppressed paclitaxel-induced mechanical ($F_{4,21} = 11.19, P < 0.0001$; Fig. 7A) and cold ($F_{4,21} = 12.61, P < 0.0001$; Fig. 7B) alldynia in a dose-dependent manner. Low-dose CP55,940 (0.3 mg/kg, i.p.) failed to attenuate paclitaxel-evoked alldynia relative to vehicle ($P > 0.84$ for both modalities) in CB1KO mice.
Higher doses of CP55,940 (3 or 10 mg/kg, i.p.) fully reversed comparisons) and preinjection levels (8B) hypersensitivities relative to vehicle (CB1KO mice on any day tested (A and B). P

Chronic high-dose CP55,940 suppressed paclitaxel-induced allodynia in CB1KO mice. In CB1KO mice, chronic high-dose treatment with CP55,940 (3 mg/kg daily, i.p.) reversed paclitaxel-induced mechanical and cold allodynia in CB1KO mice relative to vehicle (P < 0.0001) and normalized responses to prepaclitaxel levels (P > 0.49; Fig. 7, A and B). WT mice treated with CP55,940 (3 mg/kg, i.p.) exhibited severe catalepsy and thus were not used for the assessments of mechanical and cold allodynia.

Chronic High-Dose CP55,940 Suppressed Paclitaxel-Induced Allodynia in CB1KO Mice. In CB1KO mice, chronic high-dose treatment with CP55,940 (3 mg/kg daily, i.p.) reversed paclitaxel-induced mechanical and cold allodynia and thus were not used for the assessments of mechanical and cold allodynia.

Challenge in CB1KO mice receiving chronic treatment with either CP55,940 (3 mg/kg daily, i.p.) or vehicle (Fig. 8D).

Antiallodynic Effects of High-Dose CP55,940 in CB1KO Mice Were Mediated by CB2 Receptors. In paclitaxel-treated CB1KO mice, the antiallodynic effects of high-dose CP55,940 (3 mg/kg daily, i.p.) on mechanical (F3,24 = 59.44, P < 0.0001; Fig. 9A) and cold (F3,17 = 59.44, P < 0.0001; Fig. 9B) hypersensitivities were blocked by the CB2 antagonist AM630 (5 mg/kg, i.p.) at all time points (P < 0.0001). AM630 (5 mg/kg, i.p., alone did not alter mechanical or cold responsiveness relative to vehicle (P = 1.00 for all comparisons) in CB1KO mice (Fig. 9, A and B).

Discussion
Psychoactive effects have represented significant hurdles for advancing cannabinoids as pharmacotherapies (Dhopheshwarkar and Mackie, 2014). Tolerance develops to CB1-mediated locomotor effects of CP55,940 and Δ9-tetrahydrocannabinol (Δ9-THC) and is accompanied by downregulation of cannabinoid receptor binding sites in the absence of nerve injury (Oviedo et al., 1993). If tolerance develops differentially to psychotropic effects and therapeutic effects of cannabinoids, the clinical potential of these agents would be enhanced. A better understanding of the receptor mechanisms underlying the therapeutic and side effect profiles of cannabinoids observed with chronic dosing may improve current pharmacotherapies and validate novel targets (Ben Amar, 2006; Pertwee, 2009). In the present study, we used CP55,940, a potent synthetic cannabinoid with equal affinities for mouse CB1 and CB2 receptors in vitro (Abood et al., 1997; Griffin et al., 2000), and challenge in CB1KO mice receiving chronic treatment with either CP55,940 (3 mg/kg daily, i.p.) or vehicle (Fig. 8D).

Fig. 4. Chronic low-dose CP55,940 produced transient hypothermia in WT but not CB1KO mice. (A) CP55,940 (0.3 mg/kg daily, i.p. × 8 days) decreased body temperature in paclitaxel-treated WT mice on treatment day 2 but not day 7. (B) CP55,940 (0.3 mg/kg daily, i.p. × 8 days) did not alter body temperature in paclitaxel-treated CB1KO mice. Veh, vehicle. Data are expressed as mean ± S.E.M. (n = 4–6 per group). *P < 0.05 versus vehicle, two-tailed t test.
knockout mice to study the pharmacological effects associated with activation of distinct cannabinoid receptor subtypes in a model of paclitaxel-induced neuropathic pain. CP55,940 at a dose of 0.3 mg/kg i.p. suppressed paclitaxel-induced allodynia and produced undesirable side effects (i.e., hypothermia and physical withdrawal) through activation of CB1 receptors. Interestingly, we unmasked a novel CB2-mediated component of CP55,940-induced antiallodynic effects through the use of CB1KO mice. CP55,940 at a dose of 3 mg/kg i.p. (i.e., 10 times higher than the dose producing CB1-mediated pharmacological effects in WT mice) activated CB2 receptors and produced antiallodynic effects in CB1KO mice. This desirable therapeutic profile of CB2-mediated antiallodynic efficacy was sustained after chronic dosing and was not accompanied by adverse cannabimimetic effects typical of CB1 receptor activation.

Fig. 5. Rimonabant precipitated withdrawal signs in paclitaxel-treated WT but not CB1KO mice receiving chronic low-dose CP55,940. Challenge with the CB1 antagonist rimonabant (10 mg/kg, i.p.) elicited paw tremors, headshakes, and scratching bouts in paclitaxel-treated (A) WT but not (B) CB1KO mice receiving CP55,940 (0.3 mg/kg daily, i.p. × 9 days). PTX, postpaclitaxel baseline; Veh, vehicle. Data are expressed as mean ± S.E.M. (n = 4–7 per group). *P < 0.05 versus Veh + Rim (chronic vehicle treatment and challenged by rimonabant); $P < 0.05 versus Veh + Veh (chronic vehicle treatment and challenged by vehicle), one-way analysis of variance followed by Bonferroni post hoc test or two-tailed t test.

Fig. 6. High-dose CP55,940 produced catalepsy and hypothermia in paclitaxel-treated WT but not CB1KO mice. (A and C) Acute high-dose treatment with CP55,940 (3 mg/kg, i.p.) induced (A) catalepsy and (C) hypothermia in paclitaxel-treated WT mice. (B and D) Acute high-dose treatment with CP55,940 (3 mg/kg, i.p.) did not induce (B) catalepsy or (D) hypothermia in paclitaxel-treated CB1KO mice. PTX, paclitaxel; Veh, vehicle. Data are expressed as mean ± S.E.M. (n = 4–7 per group). *P < 0.05 versus vehicle; $P < 0.05 versus CP55,940 (0.3 mg/kg, i.p.), one-way analysis of variance followed by Bonferroni post hoc test.
In our study, low-dose CP55,940 (0.3 mg/kg per day, i.p.) suppressed paclitaxel-induced allodynia in WT and CB2KO, but not in CB1KO mice, suggesting that the antiallodynic effects at this low dose were mediated solely by CB2, without a contribution from CB1 receptors. Moreover, activation of CB1 receptors was sufficient to fully reverse paclitaxel-induced neuropathic pain behaviors. Our results confirmed a previous study showing that CP55,940 at this dose produces CB1-mediated antinociception in various pain models (Sain et al., 2009). These results are in agreement with previous reports suggesting that CP55,940 produces antinociceptive effects via CB1 receptors (Lichtman and Martin, 1997; Romero et al., 2002; Choong et al., 2007; Sain et al., 2009). Mixed cannabinoid agonists can produce CB1-mediated antinociception through central and peripheral mechanisms (Fox et al., 2001; Lim et al., 2003) and suppress central

**Fig. 7.** Acute CP55,940 produced dose-dependent anti-allodynic effects in paclitaxel-treated CB1KO mice. Dose response of CP55,940 (0.3, 1, 3, and 10 mg/kg, i.p.) on paclitaxel-evoked (A) mechanical and (B) cold allodynia in CB1KO mice. BL, prepaclitaxel baseline; PTX, postpaclitaxel baseline; Veh, vehicle. Data are expressed as mean ± S.E.M. (n = 4–7 per group). ∗P < 0.05 versus vehicle; †P < 0.05 versus CP55,940 (1 mg/kg, i.p.), one-way analysis of variance followed by Bonferroni post hoc test.

**Fig. 8.** In CB1KO mice, high-dose CP55,940 suppressed paclitaxel-induced allodynia without producing hypothermia or rimonabant-elicited precipitated withdrawal symptoms. (A and B) CP55,940 (3 mg/kg day, i.p./C2/C8 days) reversed paclitaxel-induced (A) mechanical and (B) cold allodynia in CB1KO mice. (C and D) CP55,940 (3 mg/kg day, i.p./C9 days) did not alter (C) body temperature or (D) produce rimonabant-elicited precipitated withdrawal symptoms in CB1KO mice. BL, pre paclitaxel baseline; PTX, postpaclitaxel baseline; Veh, vehicle. Data are expressed as mean ± S.E.M. (n = 4–7 per group). ∗P < 0.05 versus vehicle, two-tailed t test. #P < 0.05 versus prepaclitaxel baseline, repeated-measures analysis of variance.
sensitization in spinal dorsal horn neurons (Chapman, 2001) in neuropathic pain models.

CP55,940 at a 10-fold higher dose (3 mg/kg daily, i.p.) produced severe catalepsy in WT but not in CB1KO mice, consistent with previous reports on CB1-mediated catalepsy (Oviedo et al., 1993; Lichtman and Martin, 1997). Cataleptic effects of CP55,940 were absent in CB1KO mice; we therefore used CP55,940 in conjunction with CB1KO mice as a tool for studying CB2-mediated signaling in isolation from CB1 receptors (i.e., without the confounding effects of the drug on motor behavior). CP55,940 administered at the higher dose produced antiallodynic effects in CB1KO mice. Moreover, these antiallodynic effects were blocked by the CB2 antagonist AM630, suggesting that CP55,940 at the higher dose engages CB2 receptors to produce antiallodynic effects. Our results are in line with a previous study showing that both CB1 and CB2 receptors are involved in the actions of CP55,940 on acute pain and spinal nerve ligation-induced neuropathic pain (Scott et al., 2004). Activation of spinal and/or peripheral CB2 receptors by CB2 agonists, after acute or chronic administration, suppresses neuropathic pain (Yamamoto et al., 2008; Hsieh et al., 2011; Landry et al., 2012; Deng et al., 2015). CB2 agonists are likely to suppress neuropathic nociception by downregulation of proinflammatory cytokines and chemokines (Klegeris et al., 2003; Eljaschewitsch et al., 2006; Wilkerson et al., 2012; Deng et al., 2015) as well as inhibition of central sensitization (Elmes et al., 2004; Nackley et al., 2004).

CP55,940 binds with similar affinity to mouse CB1 (Kd = 0.77 nM) (Abood et al., 1997) and CB2 receptors (Kd = 0.73 nM) (Griffin et al., 2000) in in vitro assays. This relationship also holds for human CB1 and CB2 receptors (Fielder et al., 1995). Interestingly, in our in vivo study, a 10-fold higher dose of CP55,940 was required to produce CB2-mediated antiallodynic efficacy relative to CB1-mediated antiallodynic efficacy. Thus, low-dose CP55,940 preferentially engaged CB1-mediated processes, consistent with the high expression levels of CB1 compared with CB2 in the CNS (Galiigue et al., 1995; Schatz et al., 1997; Marsisano and Lutz, 1999; Onaivi et al., 2006). At a higher dose, CP55,940 suppresses pain by triggering CB2 signaling in addition to the CB1-mediated mechanism. Indeed, in an inflammatory pain model, CB2 receptors are involved in the peripheral antihyperalgesic actions of a mixed CB1/CB2 agonist WIN55,212-2 [(R)–(+)-(2,3-dihydro-5-methyl-3-(4-morpholinylnethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalen-yl-methanone], when administered locally in the paw, under conditions in which central CB1 receptors would not be activated (Nackley et al., 2003). We postulate that the higher dose of systemic CP55,940 activates both CB1 and CB2 receptors, but the beneficial antinociceptive effects (mediated by both CB1 and CB2) are masked by CB1-mediated cataleptic effects.

As expected, CP55,940 produced hypothermia in WT, but not CB1KO mice (McGregor et al., 1996; Rawls et al., 2002; Varvel et al., 2005). By contrast, chronic CP55,940, at a dose that produced CB2-mediated antiallodynic efficacy, failed to decrease body temperature in CB1KO mice, documenting that prolonged activation of CB2 receptors does not result in hypothermia (Malan et al., 2001; Valenzano et al., 2005; Yao et al., 2009; Elliott et al., 2011; Kinsey et al., 2011; Amenta et al., 2012). Chronic CP55,940-treated WT, but not CB1KO mice, showed profound withdrawal signs when challenged with the CB2 antagonist rimonabant, suggesting precipitation at CB2 receptors produces withdrawal symptoms (Tsou et al., 1995; Aceto et al., 1996; Cook et al., 1998; Rubino et al., 1998; Lichtman et al., 2001). Interestingly, although rimonabant challenge preferentially increased scratching bouts in mice treated with CP55,940 compared with vehicle, rimonabant-elicited scratching was notably absent in CB1KO mice, demonstrating that antagonist-induced scratching (analogous to pruritis [Proietto et al., 2010]) in the absence of chronic cannabinoid dosing is mediated by CB1 receptors, rather than an off-target effect of rimonabant. Our studies are the first to evaluate possible signs of physical dependence in animal pain models associated with repeated systemic activation of CB2 receptors [present data and (Deng et al., 2015)]. These studies provide strong evidence that activation of CB2 receptors produce substantial antiallodynic efficacy independent of CB1 receptors.

One of the common features of G-protein–coupled receptors is that prolonged exposure to their agonists lead to the development of tolerance (Taylor and Fleming, 2001; Martin et al., 2004). A striking observation of our study was that tolerance to the therapeutic effects of CP55,940 (0.3 mg/kg daily, i.p.) occurred later than tolerance to its psychotropic effects. Our results, along with published reports (Bass and Martin, 2000; McKinney et al., 2008; Nguyen et al., 2012), suggested that the time course of tolerance may vary between different CB1-mediated pharmacological effects (e.g., analgesia,
hypothesia, hypoaactivity). Interestingly, using the same mouse model of paclitaxel-induced neuropathy, complete tolerance developed to both antinoceptive and hyperemic effects of the prototypical cannabinoid Δ⁹-THC over 8 days (Deng et al., 2015). CP55,940 and Δ⁹-THC differ in potency at CB₁ receptors (Wiley et al., 1995; Darmani et al., 2007) and tolerance development (De Vry et al., 2004). Ligand-dependent differences in tolerance for different pharmacological effects may be attributed to the different signaling pathways recruited by CB₁ receptors (Martin et al., 2004) and regionally specific differences in receptor density and/or efficacy (Oviedo et al., 1993; McKinney et al., 2008). For instance, CP55,940 and Δ⁹-THC differ in CB₂ receptor internalization (Hisieh et al., 1999) and inhibition of adenylyl cyclase activity (Sim et al., 1995; Children and Deadwyler, 1996; Fan et al., 1996; Breivogel et al., 1998; Rubino et al., 2000a,b), whereas these ligands act similarly with respect to other CB₁ signaling pathways, such as extracellular-signal-regulated kinase activation (Rubino et al., 2005; Daigle et al., 2008). More studies that correlate behavior observations and functional signaling changes are needed to understand tolerance associated with CB₁ receptors. In addition to CB₁-associated tolerance, the present report also asked whether the high-dose CP55,940 treatment in CB₁KO mice would produce tolerance to CB₂-mediated antiallodynic effects. Notably, no decrement in CB₂-mediated antiallodynic effects was observed in CB₁KO mice treated with daily administration of the high-dose CP55,940, similar to previous reports with CB₂-prefering agonists (Yao et al., 2009; Deng et al., 2015).

In conclusion, the present report demonstrated distinct roles of cannabinoid receptor subtypes in modulating the beneficial and adverse effects of CP55,940 in an animal model of paclitaxel-induced neuropathy. CP55,940 suppressed the maintenance of paclitaxel-induced neuropathic pain through both CB₁- and CB₂-dependent mechanisms. CB₂-mediated antinoceptive effects were engaged at doses approximately 10 times higher than those required to produce CB₁-mediated antinoception. On the other hand, CB₁ but not CB₂ receptors were engaged in CP55,940-produced hypothermia, catalepsy, and cannabimimetic physical withdrawal. Our results further demonstrate that CB₂ receptors represent a potential therapeutic target for effectively and safely managing chemotherapy-induced neuropathic pain without unwanted effects.

Authorship Contributions

Participated in research design: Deng, Mackie, Hohmann.

Conducted experiments: Deng, Cornett.

Performed data analyses: Deng.

Wrote or contributed to the writing of the manuscript: Deng, Mackie, Hohmann.

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Darmani NA, Jamoyan JJ, Crivt J, and Ramirez J (2007) CB₁ cannabinoid receptor-mediated antinociception is reduced by daily administration of the high-dose CP55,940, similar to previous reports with CB₂-prefering agonists (Yao et al., 2009; Deng et al., 2015).

In conclusion, the present report demonstrated distinct roles of cannabinoid receptor subtypes in modulating the beneficial and adverse effects of CP55,940 in an animal model of paclitaxel-induced neuropathy. CP55,940 suppressed the maintenance of paclitaxel-induced neuropathic pain through both CB₁- and CB₂-dependent mechanisms. CB₂-mediated antinoceptive effects were engaged at doses approximately 10 times higher than those required to produce CB₁-mediated antinoception. On the other hand, CB₁ but not CB₂ receptors were engaged in CP55,940-produced hypothermia, catalepsy, and cannabimimetic physical withdrawal. Our results further demonstrate that CB₂ receptors represent a potential therapeutic target for effectively and safely managing chemotherapy-induced neuropathic pain without unwanted effects.

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