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Antinociceptive effects of the non-selective cannabinoid receptor agonist CP 55,940 are absent in CB1$^{-/-}$ and not CB2$^{-/-}$ mice in models of acute and persistent pain

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ABSTRACT

Previous studies have suggested a role for both CB1 and CB2 cannabinoid receptors in modulation of nociception. To further examine the role of CB1 and CB2 receptors in antinociception, we evaluated the efficacy of the non-selective cannabinoid receptor agonist, CP 55,940, in models of acute, inflammatory, and neuropathic pain in control mice, CB1 receptor knockout mice, and CB2 receptor knockout mice. In control C57BL/6 mice, administration of CP 55,940 (0.03–0.3 mg/kg, i.p.) reversed complete Freund's adjuvant-induced tactile allodynia, reversed tactile allodynia in the spinal nerve ligation model and inhibited the noxious heat-evoked tail withdrawal response. In addition to its antinociceptive effects, CP 55,940 produced an impairment of motor coordination in the rotarod test. The antinociceptive effects produced by CP 55,940 and associated motor deficits were found to be completely abolished in CB1 receptor knockout mice. In contrast, the antinociceptive effects of CP 55,940 in all pain models were fully retained in CB2 receptor knockout mice, along with the associated motor deficits. The results suggest that the antinociceptive effects of CP 55,940 in models of acute and persistent pain, along with the associated motor deficits, are mediated by CB1 receptors, and likely not CB2 receptors.

1. Introduction

Cannabinoid receptor agonists have been shown to be effective in animal models of pain by inhibiting acute nociceptive responses, as well as reversing the behavioral hypersensitivity that occurs from chemical stimuli, peripheral inflammation, or neuropathy (for review, see Pertwee, 2001; Whiteside et al., 2007). While cannabinoid receptor agonists have been shown to produce antinociception in a variety of animal pain models, efficacy is typically associated with significant side effects, including catalepsy, motor deficits, and hypothermia (Herzberg et al., 1997; Ledent et al., 1999; Romero et al., 2002; Smith et al., 1994). It has been suggested that these adverse effects, in addition to the antinociceptive efficacy of cannabinoid receptor agonists, occur as a consequence of CB1 receptor activation in the central nervous system (CNS) (Ledent et al., 1999; Lichtman and Martin, 1997; Martin et al., 1993, 1999; Richardson et al., 1998; ; Zimmer et al., 1999).

In contrast to CB1 receptors, CB2 receptors are found primarily in peripheral immune cells (Facci et al., 1995; Galiegue et al., 1995; Rice et al., 2002), and several reports have demonstrated antinociceptive efficacy of selective CB2 receptor agonists in models of acute, inflammatory and neuropathic pain (Bridges et al., 2001; Elmes et al., 2005; Ibrahim et al., 2003; Quartilho et al., 2003; Scott et al., 2004; Valenzano et al., 2005). Results from studies such as these have suggested that selective CB2 receptor agonists may produce antinociception without exhibiting significant side effects, and support the potential development of selective CB2 receptor agonists as a viable alternative to non-selective cannabinoid agonists for the treatment of pain.

Since reports in the literature support a role for both CB1 and CB2 receptors in antinociception, non-selective cannabinoid receptor agonists may be used as a tool to investigate the contribution of each cannabinoid receptor subtype to antinociceptive efficacy. Commonly used non-selective cannabinoid receptor agonists include CP 55,940 and WIN 55,212-2. CP 55,940 displays similar affinity for both CB1 and CB2 receptors (Felder et al., 1995), and several studies have described the antinociceptive action of CP 55,940 in rat pain models (Fox et al., 2001; Lichtman and Martin, 1997; Romero et al., 2002; Scott et al., 2004). One utility of mouse pain models is that antinociceptive mechanisms of action may be investigated using transgenic or knockout mice. In the present study, we examined the antinociceptive effects of CP 55,940 in acute and persistent pain models in C57BL/6 mice, and also

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determined effects on motor coordination. Additionally, to investigate the cannabinoid receptor subtypes involved in mediating antinociception, the effects of CP 55,940 were evaluated in CB1 receptor knockout ($CB_1^{-/-}$), CB2 receptor knockout ($CB_2^{-/-}$), and wild type littermate control mice ($CB_1^{+/+}$, $CB_2^{+/+}$).

2. Materials and methods

2.1. Animals

Mice were maintained in a climate-controlled room on a 12:12 h light/dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Merck & Co., Inc., West Point, PA and were in accordance with *The Guide for the Care and Use of Laboratory Animals*. C57BL/6 mice were obtained from Taconic Farms (Germantown, NY). Breeding pairs of mice heterozygous for the CB1 receptor gene ($CB_1^{+/-}$) were obtained from Andreas Zimmer (University of Bonn, Bonn). Breeding pairs of mice heterozygous for the CB2 receptor gene ($CB_2^{+/-}$) were obtained from Deltagen (San Mateo, CA). Breeding and genotyping occurred at Taconic Farms (Germantown, NY) to generate CB1 knockout ($CB_1^{-/-}$), CB2 knockout ($CB_2^{-/-}$) and wild type ($CB_1^{+/+}$, $CB_2^{+/+}$) mice for behavioral testing. All mice were 20–40 g at the time of behavioral testing.

2.2. Complete Freund's Adjuvant (CFA) model of inflammatory pain

Mice were placed in individual plastic cylinders on an elevated mesh platform and allowed to acclimate. Baseline mechanical sensitivity was determined by applying a series of logarithmically-spaced calibrated von Frey filaments (0.02–2 g, Stoelting, Wood Dale, IL) to the plantar surface of the left hind paw. A positive response was indicated by a sharp withdrawal of the hind paw. The median mechanical withdrawal threshold was determined by using the nonparametric method of Chaplan et al. (1994) in which the responses to the next four filaments following the determination of a crossover threshold (i.e. from response to no response, or vice versa) were recorded. Following assessment of baseline thresholds, mice received 30 μl of 50% CFA (1:1 Complete : Incomplete Freund's Adjuvant) into the plantar surface of the left hind paw. Twenty-four hours following hind paw injection of CFA, mice were visually examined for edema of the affected paw and were tested for tactile allodynia using von Frey filaments, as previously described. To examine effects of CP 55,940 on tactile allodynia, mice received either drug or vehicle, and paw withdrawal thresholds were recorded at 30 min post-administration.

2.3. Spinal Nerve Ligation (SNL) model of neuropathic pain

Baseline paw withdrawal thresholds in response to stimulation with von Frey filaments were obtained for all mice prior to surgery, using the method previously described in the CFA model. Neuropathy was produced using a modified spinal nerve ligation procedure (Kim and Chung, 1992). Under gaseous anesthesia using isoflurane, the L5 spinal nerve was tightly ligated with braided 6-0 silk thread approximately 5 mm distal to the DRG. Wounds were closed in layers and mice were allowed to recover. Mice were assessed for tactile allodynia seven days following nerve ligation using von Frey filaments, as previously described. To examine effects of CP 55,940 on tactile allodynia, mice received either drug or vehicle following determination of post-ligation thresholds, and paw withdrawal thresholds were recorded at 30 min post-administration.

2.4. Tail withdrawal test

Following acclimation to the laboratory, mice were tested for baseline tail withdrawal response latencies by immersing the distal portion of the tail into a 49 °C warm water bath (Lindberg/Blue), and the time between the initiation of the stimulus and the rapid removal of the tail from the water bath was recorded (secs). The baseline tail withdrawal latency was recorded by determining the average of two tail withdrawal responses, separated by approximately 10 s. A cutoff of 15 s was employed to avoid tissue damage. To examine effects of CP 55,940 on acute noxious heat sensitivity, mice received either drug or vehicle following determination of baseline tail withdrawal latencies, and tail withdrawal latencies were recorded in duplicate at 30 min post-administration.

2.5. Rotarod test of motor coordination

Mice were trained to remain on a rotarod apparatus (IITC Inc., Woodland Hills, CA) revolving at 12 rpm for 120 s. Mice were given two attempts to stay on the rotarod, and mice that could not remain on the rotarod during the training session were excluded from the study. Mice that successfully completed the training were placed on an accelerating rotarod (increasing in speed from 4 to 33 rpm during a 300-sec-period) and the length of time that the mice remained on the rotarod was recorded, up to a maximum of 300 s. To examine effects of CP 55,940 on motor coordination, drug or vehicle was administered following training, but 30 min before mice were placed on the accelerating rotarod.

2.6. Data analysis

All data are represented as mean ± SEM. Differences in responses from vehicle treatments were compared by *t*-tests for single dose comparisons, and by one-way ANOVA followed by Dunnett's post-hoc test for multiple dose comparisons. Effects of treatment over time were compared using two-way repeated measures ANOVA (time × dose) with post-hoc Tukey's test. SigmaStat was used for all statistical analyses and significance was defined as $p < 0.05$.

2.7. Drugs and chemicals

Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA) were purchased from Sigma (St. Louis, MO). CP 55,940 was purchased from Tocris (Ellisville, MO), dissolved in 25% dimethyl sulfoxide (DMSO) in normal saline and administered i.p. in a volume of 10 mL/kg. In all models, CP 55,940 was administered 30 min before behavioral testing.

3. Results

3.1. Behavioral phenotype of C57BL/6, $CB_1^{+/+}$, $CB_2^{+/+}$, $CB_1^{-/-}$, and $CB_2^{-/-}$ mice in models of nociception and motor coordination

Behavioral phentotyping of all mice was performed by determining: a) baseline sensitivity to von Frey filament stimulation, b) tactile allodynia following CFA injection, c) tactile allodynia following SNL, d) sensitivity to warm water tail immersion, and e) motor coordination on the rotarod. As summarized in Table 1, the phenotypes of control C57BL/6, $CB_1^{+/+}$, $CB_2^{+/+}$, $CB_1^{-/-}$, and $CB_2^{-/-}$ mice were similar in all tests of nociception. In terms of motor coordination, all groups of mice were similar with the exception of $CB_1^{-/-}$ mice, which displayed a modest, but significantly reduced time on the rotarod.

3.2. Effect of CP 55,940 on nociception and motor coordination in C57BL/6 mice

Dose-response functions were generated in C57BL/6 mice using doses of 0.03, 0.1 and 0.3 mg/kg of CP 55,940 (i.p.) in all pain models and in the rotarod test. Intra-plantar injection of CFA produced tactile allodynia to von Frey filament stimulation 24 h following the injection (Fig. 1A). Administration of CP 55,940 (i.p.) dose-dependently reversed the CFA-induced tactile allodynia and increased mechanical withdrawal thresholds beyond baseline values at the highest dose tested 30 min post-administration, at the time of maximal effect

Table 1
Behavioral phenotype of C57BL/6, $CB_1^{+/+}$, $CB_2^{+/+}$, $CB_1^{-/-}$, and $CB_2^{-/-}$ mice in models of nociception and motor coordination. Data are expressed as mean +/− SEM. $^*p < 0.05$ vs. $CB_1^{+/+}$

Behavioral Test	C57BL/6	$CB_1^{+/+}$	$CB_1^{-/-}$	$CB_2^{+/+}$	$CB_2^{-/-}$
Mechanical withdrawal threshold (g)					
Baseline	0.87 ± 0.03	0.82 ± 0.03	0.87 ± 0.03	0.91 ± 0.06	0.84 ± 0.03
Post-CFA	0.11 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Post-SNL	0.13 ± 0.02	0.13 ± 0.02	0.18 ± 0.02	0.10 ± 0.01	0.10 ± 0.02
Tail withdrawal latency (sec.)	2.19 ± 0.09	2.17 ± 0.11	2.36 ± 0.22	3.98 ± 0.23	3.76 ± 0.18
Rotarod Latency (sec.)	296.2 ± 2.7	297.4 ± 1.6	270.8 ± 4.3 *	283.5 ± 4.9	286.2 ± 18.7

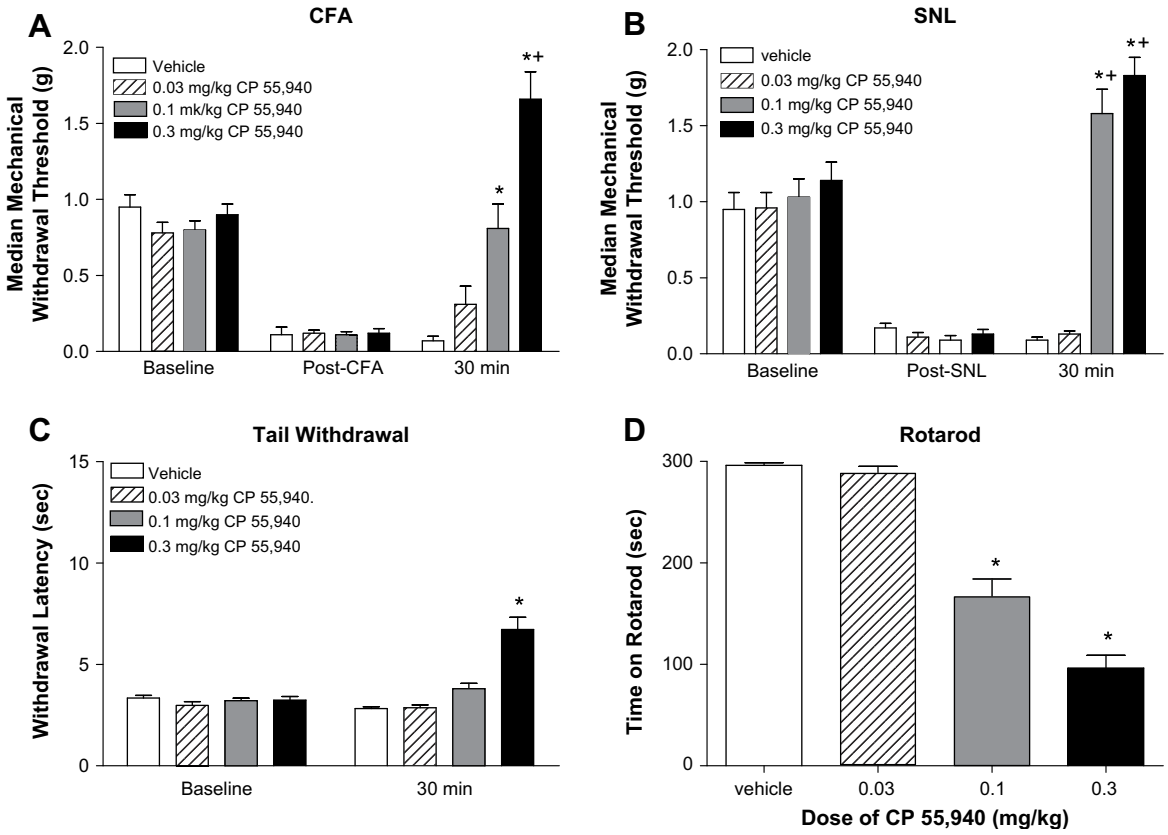

Fig. 1. Effect of CP 55,940 in C57BL/6 mice. Systemic (i.p.) administration of CP 55,940 (A) dose-dependently reversed tactile allodynia in the CFA model (B) dose-dependently reversed SNL-induced tactile allodynia (C) increased tail withdrawal latencies at the highest dose used and (D) dose-dependently inhibited motor coordination. $^{*}p < 0.05$ compared to vehicle treatment at 30 min; $+p < 0.05$ compared to baseline; $n = 6$–12 per group.

(0.3 mg/kg) (Fig. 1A). Additionally, CP 55,940 was found to increase paw withdrawal thresholds to von Frey stimulation in naïve mice, with the maximum dose of 0.3 mg/kg increasing withdrawal thresholds from 0.9 ± 0.05 g to 1.8 ± 0.11 g ($p < 0.05$; data not shown). Spinal nerve ligation (SNL) produced tactile allodynia in C57BL/6 mice when measured 7 days following the surgical procedure (Fig. 1B). The spinal nerve injury-induced tactile allodynia was dose-dependently reversed by CP 55,940 beyond baseline values 30 min post-administration, at the time of maximal effect (Fig. 1B). In the thermal tail withdrawal test, i.p. administration of CP 55,940 produced a significant increase in tail withdrawal latency 30 min following administration only at the highest dose of 0.3 mg/kg (Fig. 1C). In addition to its antinociceptive effects, CP 55,940 produced dose-dependent cataleptic-like behavior in the mice. Mice treated with 0.03 and 0.1 mg/kg CP 55,940 were more easily startled and likely to jump when startled, and mice receiving the highest dose of 0.3 mg/kg exhibited spontaneous cataleptic-like behavior (e.g. immobility). Thus, to examine whether the cataleptic-like behavior could be associated with deficits in motor coordination, effects of CP 55,940 were also evaluated in the rotarod test. Administration of CP 55,940 dose-dependently inhibited motor coordination in C57BL/6 mice with significant deficits in motor function observed at the 0.1 and 0.3 mg/kg doses 30 min post-administration (Fig. 1D). In all models, treatment with vehicle did not affect the responses of mice (Fig. 1A–D).

3.3. Effect of CP 55,940 on nociception and motor coordination in $CB_1^{-/-}$ and $CB_1^{+/+}$ mice

The effects of CP 55,940 on nociceptive thresholds and motor coordination were determined in $CB_1^{-/-}$ and $CB_1^{+/+}$ mice by administering a dose of 0.3 mg/kg, which produced the greatest effects in control C57BL/6 mice. Twenty-four hours following administration of CFA, $CB_1^{-/-}$ mice developed tactile allodynia that was similar in magnitude to that of wild type littermate control and C57BL/6 mice (Fig. 2A, Table 1). Administration of CP 55,940 produced a significant reversal of CFA-induced tactile allodynia beyond baseline withdrawal thresholds in $CB_1^{+/+}$ mice, but had no effect on tactile allodynia in $CB_1^{-/-}$ mice (Fig. 2A). Seven days following spinal nerve ligation, $CB_1^{-/-}$ mice developed tactile allodynia that was similar in magnitude to that of $CB_1^{+/+}$ and C57BL/6 mice (Fig. 2B, Table 1). Administration of CP 55,940 produced a significant reversal of the spinal nerve ligation-induced tactile allodynia in $CB_1^{+/+}$ mice beyond baseline, but had no significant effect in $CB_1^{-/-}$ mice (Fig. 2B). CP 55,940 also produced a significant increase in tail withdrawal latencies in $CB_1^{+/+}$ mice, but did not affect withdrawal latencies in $CB_1^{-/-}$ mice (Fig. 2C). Additionally, administration of CP 55,940 in $CB_1^{+/+}$ mice produced a significant inhibition of motor coordination in the rotarod test similar to control C57BL/6 mice, whereas administration of CP 55,940 in $CB_1^{-/-}$ mice did not significantly affect motor coordination compared to vehicle treated mice (Fig. 2D). In all models, treatment with vehicle did not affect the responses of mice ($p > 0.05$; Fig. 2A–D).

3.4. Effect of CP 55,940 on nociception and motor coordination in $CB_2^{-/-}$ and $CB_2^{+/+}$ mice

Similar to the protocol used in evaluating $CB_1^{-/-}$ and $CB_1^{+/+}$ mice, the effects of CP 55,940 on nociceptive thresholds and motor coordination were determined in $CB_2^{-/-}$ and $CB_2^{+/+}$ mice by administering the maximum dose of 0.3 mg/kg. Twenty-four hours following

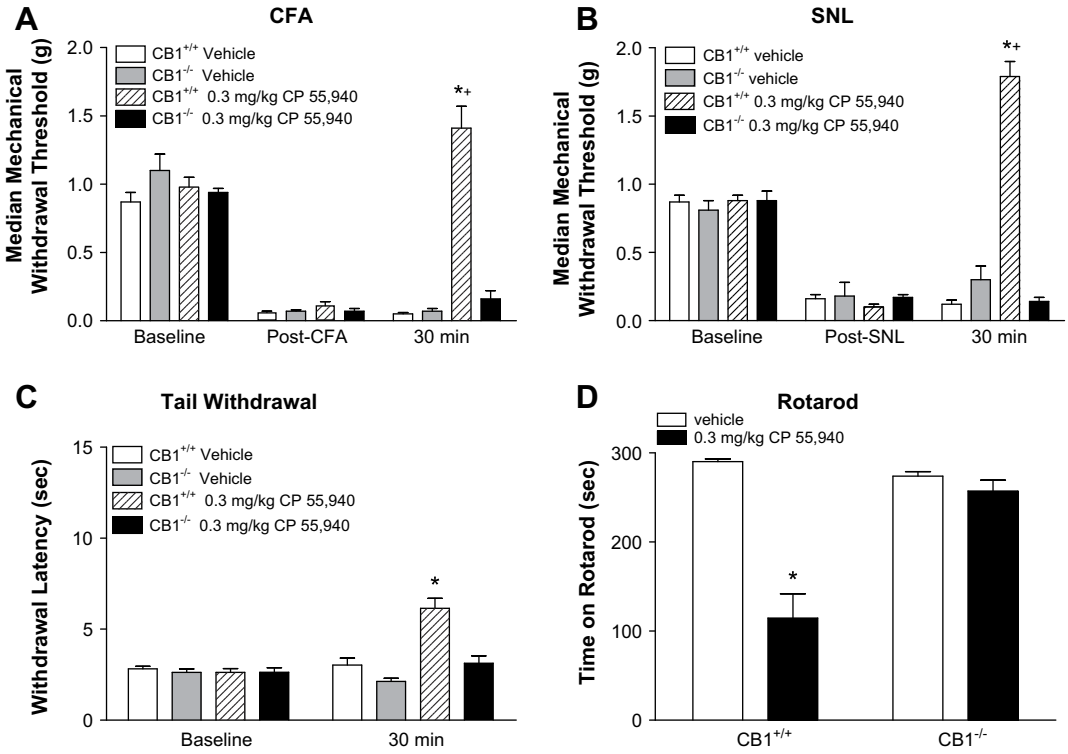

Fig. 2. Effect of CP 55,940 in $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. Systemic (i.p.) administration of 0.3 mg/kg CP 55,940 (A) reversed CFA-induced tactile allodynia in $CB1^{+/+}$, but not $CB1^{-/-}$ mice (B) reversed SNL-induced tactile allodynia in $CB1^{+/+}$, but not $CB1^{-/-}$ mice (C) increased tail withdrawal latencies in $CB1^{+/+}$, but not $CB1^{-/-}$ mice and (D) inhibited motor coordination in $CB1^{+/+}$, but not $CB1^{-/-}$ mice. $^*p < 0.05$ compared to vehicle treatment at 30 min; $+p < 0.05$ compared to baseline; $n = 6$–13 per group.

administration of CFA, $CB_2^{-/-}$ mice developed tactile allodynia that was similar in magnitude to that of wild type littermate control and C57BL/6 mice (Fig. 3A, Table 1). In both $CB_2^{+/+}$ and $CB_2^{-/-}$ mice, administration of CP 55,940 produced a similar, significant reversal of CFA-induced tactile allodynia, and an increase in withdrawal thresholds beyond baseline values which was similar to that observed in $CB_1^{+/+}$ and C57BL/6 mice (Fig. 3A). Seven days following spinal nerve ligation, $CB_2^{+/+}$ and $CB_2^{-/-}$ mice displayed a similar magnitude of tactile allodynia that was also similar to the tactile allodynia observed in $CB_1^{+/+}$, $CB_1^{-/-}$, and control C57BL/6 mice (Fig. 3B, Table 1). Administration of CP 55,940 produced a significant reversal of SNL-induced tactile allodynia in both $CB_2^{+/+}$ and $CB_2^{-/-}$ mice that was similar in magnitude, and which resulted in withdrawal thresholds beyond baseline thresholds similar to that observed in $CB_1^{+/+}$ and control C57BL/6 mice (Fig. 3B). In the warm water tail withdrawal test, administration of CP 55,940 produced a significant increase in tail withdrawal latencies which was similar in both $CB_2^{+/+}$ and $CB_2^{-/-}$ mice (Fig. 3C) and did not differ in magnitude compared to post-CP 55,940 latencies in C57BL/6 and $CB_1^{+/+}$ mice. In the rotarod test, administration of CP 55,940 resulted in a similar significant impairment of motor coordination in both $CB_2^{+/+}$ and $CB_2^{-/-}$ mice compared to vehicle treated controls (Fig. 3D). In all models, treatment with vehicle did not affect the responses of mice ($p > 0.05$; Fig. 3A–D).

4. Discussion

The results from the present study suggest that the antinociceptive effects and impaired motor coordination following administration of the non-selective cannabinoid receptor agonist CP 55,940 in mice are mediated by CB1, and likely not CB2 receptors. CP 55,940 is a cannabinoid receptor agonist that has been shown to have similar binding affinity (CB1 Ki = 3.7 nM; CB2 Ki = 2.5 nM) and potency (CB1 cAMP IC_{50} = 1.8 nM; CB2 cAMP IC_{50} = 2.9 nM) for CB1 and CB2 receptors in stably transfected cell lines (Felder et al., 1995). In the present study, administration of CP 55,940 consistently produced similar antinociceptive effects in models of acute, inflammatory and neuropathic pain in control C57BL/6, $CB_1^{+/+}$, $CB_2^{+/+}$, and $CB_2^{-/-}$ mice, but failed to produce antinociception in $CB_1^{-/-}$ mice. The highest doses of CP 55,940 both reversed injury-induced tactile allodynia and increased withdrawal thresholds beyond baseline values, and these effects on tactile allodynia and acute nociception were completely absent in $CB_1^{-/-}$ mice, implicating CB1 receptors in both types of antinociceptive action. Moreover, impairment of motor coordination following administration of CP 55,940 was consistently observed in control C57BL6, $CB_1^{+/+}$, $CB_2^{+/+}$, and $CB_2^{-/-}$ mice, but was absent in $CB_1^{-/-}$ mice, supporting a role for CB1 receptors in both the antinociceptive effects and motor impairment produced by CP 55,940.

That activation of CB1 receptors results in antinociception has been demonstrated in numerous studies, primarily through the use of selective cannabinoid receptor antagonists (for review, see Pertwee, 2001). In support, the antinociceptive efficacy of the non-selective cannabinoid receptor agonists, CP 55,940 and WIN 55212, has been shown to be blocked by selective CB1 receptor antagonists in models of inflammatory and neuropathic pain (Bridges et al., 2001; Fox et al., 2001; Kehl et al., 2003; Martin et al., 1999). This antinociceptive action appears to be mediated, at least in part, via spinal cord CB1 receptors, since the inhibitory action of cannabinoid agonists on spinal sensory neuron activity can be blocked by spinal application of CB1 receptor antagonists (Choong et al., 2007; Johanek and Simone, 2005; Liu and Walker, 2006). The previously established role of CB1 receptors in antinociception is further supported in the present study, as the antinociceptive efficacy of CP 55,940 was completely abolished in $CB_1^{-/-}$ mice, suggesting that activation of this receptor is sufficient for antinociceptive efficacy

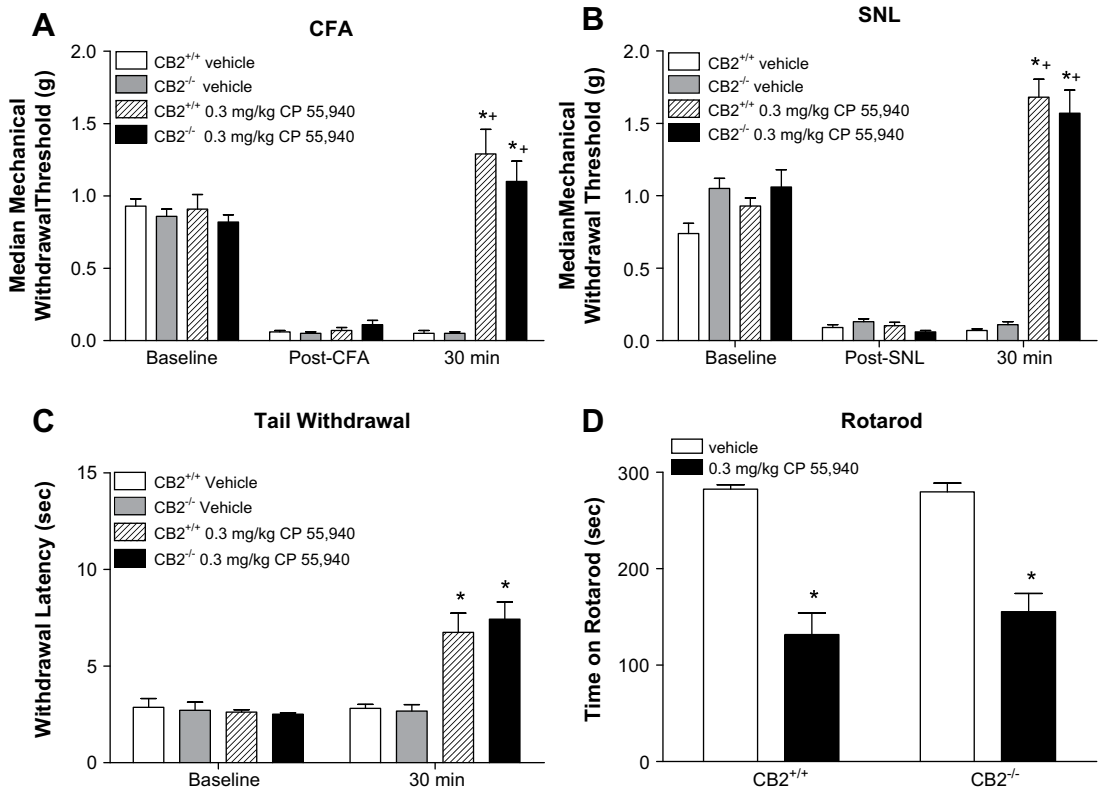

Fig. 3. Effect of CP 55,940 in $CB_2^{+/+}$ and $CB_2^{-/-}$ mice. Systemic (i.p.) administration of 0.3 mg/kg CP 55,940 (A) reversed CFA-induced tactile allodynia in both $CB2^{+/+}$ and $CB2^{-/-}$ mice (B) reversed SNL-induced tactile allodynia in both $CB2^{+/+}$ and $CB2^{-/-}$ mice (C) increased tail withdrawal latencies in both $CB2^{+/+}$ and $CB2^{-/-}$ mice and (D) inhibited motor coordination in both $CB2^{+/+}$ and $CB2^{-/-}$ mice. $^*p < 0.05$ compared to vehicle treatment; $+p < 0.05$ compared to baseline; $n = 6$–7 per group.

in models of inflammatory and neuropathic pain. Moreover, the antinociceptive effects of CP 55,940 in the tail withdrawal assay were also abolished in $CB_1^{-/-}$ mice, supporting the notion that activation of CB1 receptors is sufficient to inhibit responses to acute noxious stimuli in naïve animals (Ledent et al., 1999; Lichtman and Martin, 1997; Romero et al., 2002).

In addition to CB1 receptors, recent studies have also suggested a role for CB2 receptors in antinociception in models of acute, inflammatory and neuropathic pain (for review, see Whiteside et al., 2007). A number of reports have demonstrated that the antinociceptive action of non-selective cannabinoid receptor agonists in models of inflammatory and neuropathic pain can be inhibited by both selective CB1 and CB2 receptor antagonists (Clayton et al., 2002; Kehl et al., 2003; Scott et al., 2004). Additionally, several compounds that have been described as selective CB2 receptor agonists, including AM1241 and GW4058333, have been shown to produce antinociception in models of acute, inflammatory and neuropathic pain (Ibrahim et al., 2003; Quartilho et al., 2003; Valenzano et al., 2005; Whiteside et al., 2007). In the present study, the antinociceptive effects of CP 55,940 in models of acute, inflammatory and neuropathic pain were unaffected in $CB_2^{-/-}$ mice, suggesting that activity of this compound on CB2 receptors is not required for antinociceptive efficacy. These results are somewhat inconsistent with Ibrahim et al. (2006), who found that the antinociceptive effects of both AM1241 and WIN 55,212-2 on acute heat nociception were dramatically reduced in $CB_2^{-/-}$, but not $CB_1^{-/-}$ mice. Although the specific reasons for this discrepancy are unclear, it should be noted that there were differences in experimental design in these studies, including the use of different cannabinoid agonists and noxious stimuli. Additionally, Ibrahim et al. (2006) did not examine the efficacy of cannabinoid agonists in $CB_1^{-/-}$ and $CB_2^{-/-}$ mice in models of inflammatory or neuropathic pain.

The results from the present study suggesting that activity of CP 55,940 on CB2 receptors is not required for efficacy exemplifies the somewhat controversial role of this receptor in antinociception. For example, while the aforementioned reports support a role for CB2 receptors in antinociception, other studies have found that antinociception produced by non-selective cannabinoid agonists is blocked primarily by CB1 receptor antagonists, and not CB2 receptor antagonists (Bridges et al., 2001; Chin et al., 2007; Choong et al., 2007; Dyson et al., 2005; Liu and Walker, 2006). One likely explanation for these conflicting results is that the pharmacological profiles of commonly used CB1 and CB2 receptor ligands are not fully known, and thus potential activities at other receptor subtypes cannot be ruled out (see Begg et al., 2005). For example, although AM1241 was found to have ~80-fold selectivity for CB2 compared to CB1 receptors in a binding assay (Ibrahim et al., 2003), in-house data demonstrate that AM1241 can bind to other pain targets with Ki values less than 1 μM, including nicotinic receptors and sodium channels (data not shown). Additionally, recent results in cell-based functional assays suggest that AM1241 does not necessarily function as a full CB2 receptor agonist, and the pharmacology of AM1241 also differs from the CB2 receptor agonist GW405833 in terms of opioid involvement and effects on heat sensitivity, implying different mechanisms of action (Bingham et al., 2007; Ibrahim et al., 2006; Whiteside et al., 2005; Yao et al., 2006). Thus, the somewhat conflicting results regarding the role of CB2 receptors in antinociception are likely due, at least in part, to an incomplete understanding of the mechanisms by which identified CB2 receptor ligands produce antinociception.

In addition to effects on nociception, CP 55,940 was found to produce significant deficits in rotarod performance at antinociceptive doses in C57BL6, $CB_1^{+/+}$, $CB_2^{+/+}$, and $CB_2^{-/-}$ mice, but not $CB_1^{-/-}$ mice, suggesting that impairment of motor coordination is due to activity

on CB1 receptors. Previous reports examining impairment of motor function and catalepsy produced by cannabinoid agonists have shown that these side effects can be blocked by treatment with CB1 receptor antagonists (Lichtman and Martin, 1997; Romero et al., 2002). Moreover, studies utilizing identified CB2 receptor agonists have consistently reported a lack of psychotropic-like effects, further suggesting that CB1, and not CB2, receptors are primarily responsible for these side effects (for review, see Whiteside et al., 2007). In the present study, CP 55,940 was found to inhibit motor coordination at antinociceptive doses, raising the possibility that motor deficits may have contributed to the observed antinociceptive effects. Although deficits in motor coordination may possibly be contributing, in part, to the observed behavioral antinociceptive effects, CP 55,940 has been shown to directly affect spinal sensory transmission as well. Several studies have shown that CP 55,940 specifically inhibits activity of spinal sensory neurons via CB1 receptors, suggesting that this compound has actions on both sensory and motor physiology that can be discriminated (Choong et al., 2007; Hohmann et al., 1999; Johanek and Simone, 2005).

In the present study, behavioral phenotyping of C57BL6, $CB_1^{+/+}$, $CB_2^{+/+}$, $CB_1^{-/-}$ and $CB_2^{-/-}$ mice revealed similar basal response thresholds to acute noxious stimuli, and similar tactile allodynia following CFA injection and SNL. These results verify that tonic activation of either CB1 or CB2 receptors is limited in terms of affecting sensory thresholds in either the absence or presence of insult. The lack of tonic CB1 receptor influence on sensory thresholds is consistent with other reports that have reported a lack of phenotype of $CB_1^{-/-}$ mice in a variety of pain models (Castane et al., 2006; Ledent et al., 1999; Zimmer et al., 1999). Ibrahim et al. (2003), however, did report basal mechanical, but not heat, hypersensitivity in $CB_1^{-/-}$ mice suggesting CB1 receptor-mediated tonic inhibitory tone. One possible explanation for this discrepancy is that different background mouse strains were used in these studies (C57BL/6 vs. 129/SvJ) which could influence phenotype.

In contrast to the lack of phenotype on sensory thresholds, $CB_1^{-/-}$ mice did show a significant reduction in motor coordination on the rotarod test. These results are consistent with Zimmer et al. (1999) who reported that $CB_1^{-/-}$ mice displayed increased lack of motion in the ring catalepsy test, and reduced spontaneous activity in the open field test. Although inconsistent with the notion that CB1 agonists produce motor impairment, this discrepancy may be due to tonic vs. acute modulation of the receptor, or may be associated with an impairment of adaptation to a novel environment observed in $CB_1^{-/-}$ mice (Ledent et al., 1999).

In summary, the results from the present study demonstrate that activation of CB1 receptors is sufficient for the antinociceptive action of CP 55,940 in a variety of mouse pain models, and that activation of CB2 receptors is not required for the antinociceptive efficacy of this compound. The somewhat controversial role of CB2 receptors in antinociception is further illustrated by these results, and continued investigation of the role of CB2 receptors in nociceptive modulation will likely be facilitated through the development of more potent and selective CB2 receptor ligands.

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