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Modulation of Pain by Endocannabinoids in the Periphery

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Abstract

Activation of cannabinoid receptors using systemic treatments produces analgesia in a variety of experimental pain models, but these effects are hindered by sedation and motor impairment mediated by receptors in the central nervous system. Targeting the endocannabinoid system in the periphery can bypass these unwanted side effects while still producing analgesia in both acute and chronic pain states. This chapter discusses the different approaches to increasing peripheral endocannabinoid activity in experimental models of acute and chronic pain, including inflammatory pain, neuropathic pain, and sickle cell disease. We also explore how these treatments alter nociceptive activity in the peripheral nervous system.

Keywords: pain, hyperalgesia, nociceptors, primary afferent nerve fibers, cannabinoids

1. Introduction

Although the cannabis plant (*Cannabis sativa*) has been used as a folk remedy to treat various ailments for thousands of years, it is only within the last century that its active components have been isolated and identified. While some of its effects are well documented, its impact on pain had been less clear due to confounding effects on mood, motor impairment, and sedation. Isolation of the psychoactive components of the cannabis plant and the development of synthetic cannabinoid compounds enabled more rigorous testing. Identification of a cannabinoid receptor (CB1) in 1988 gave insight into the mechanisms of the cannabis effect, as did the discovery of endogenous ligands, referred to as endocannabinoids [1–4]. Studies

in rats showed that when applied intravenously or directly to the spinal cord, cannabinoid agonists attenuated responses to noxious mechanical and thermal stimulation in nociceptive spinal neurons [5–7]. These early studies provided the first evidence of a direct effect of cannabinoids in pain inhibition and led to further investigations to identify the mechanisms underlying cannabinoid effects on neuronal activity.

The endogenous cannabinoid system consists of two well-characterized receptor subtypes, CB1 and CB2, and their endogenous ligands, from which anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) are the most studied [8–10]. Cannabinoid receptors are G-protein coupled, mainly to $G_{i/o}$, which inhibits adenylyl cyclase [3, 11], and voltage-dependent Ca^{2+} channels [12]. CB1 receptors are expressed primarily in the nervous system, but are also present in non-neuronal tissues. CB2 receptors are mainly located peripherally, and are associated with modulation of immune cells [4, 13, 14]. Since CB receptors are widely distributed, their activation produces a wide variety of behavioral and physiological responses.

1.1. Role of the endocannabinoid system in anti-nociception and neuroprotection

Activation of cannabinoid receptors has been shown to produce anti-nociception in experimental models of inflammatory pain, including formalin [15, 16], carrageenan [17–19], CFA, complete Freund's adjuvant [20], and capsaicin [19, 21–24]. In addition, the administration of cannabinoid antagonists has been shown to enhance pain behavior in formalin and carrageenan models [15, 18], suggesting that tonic activation of cannabinoid receptors contributes to anti-nociception in response to inflammation. Systemic administration of cannabinoid agonists has also been shown to attenuate neuropathic pain following peripheral nerve injury (CCI model [25], partial sciatic nerve ligation [26], spinal nerve injury [27], L5/L6 ligation [28, 29]), diabetic neuropathy (type 1 [30–32] and type 2 [32]), and chemotherapy induced peripheral neuropathy [33–36]. In humans, cannabinoid agonists attenuated post-operative pain [37] and also enhanced the analgesic efficacy of opioids [38]. Two small clinical evaluations of the efficacy of (-) Δ^9 -tetrahydrocannabinol (THC), the main psychoactive compound of the cannabis plant, reported pain relief comparable to codeine [39, 40]. Unfortunately, higher doses tended to produce significant side effects including sedation, dizziness, ataxia and blurred vision.

In addition to anti-nociception, the endocannabinoid system has a neuroprotective function. In a model of cerebral ischemia, cannabinoid agonists, cannabidiol and THC attenuated toxicity related to the activity of excitatory neurotransmitters in the rat cerebral cortex independent from CB1 and CB2 receptors [41]. Cannabidiol is known to have low affinity for cannabinoid receptors, and has also been shown to act as a negative allosteric modulator at the CB1 receptor and a reverse agonist at the CB2 receptor [42, 43]. Another study reported the involvement of CB1 receptors in the reduction of neuronal loss [44]. Further, an *in vitro* study of hypoxic ischemia demonstrated a possible role for CB2 receptors [45]. The endogenous cannabinoid ligand, 2-AG, was shown to be neuroprotective in a model of traumatic brain injury, resulting in reduced edema and neuronal loss in the hippocampus [46]. Endocannabinoids have also been shown to protect against neurodegenerative diseases, including Alzheimer's disease, where the inhibition of microglial activation may prevent pathological changes associated with beta amyloid [47]. There is also evidence that cannabinoids possess antioxidant

properties through the activity of cannabinoid receptors located on microglia, astrocytes, and other immune cells, where activation inhibits the release of pro-inflammatory substances [48–54]. Increased expression of CB2 on microglia and astrocytes has been observed in the area of lesion [54]. The administration of a CB2 agonist slowed the progression of amyotrophic lateral sclerosis in mice, and the activation of the endocannabinoid system protected against myelin degeneration in multiple sclerosis through a combination of immunosuppression and neuroprotection [55–57]. In studies of peripheral neuropathy produced by chemotherapy, WIN 55,212-2 prevented the development of neuropathy induced by cisplatin treatment [33], and when WIN 55,212-2 treatment was initiated after sciatic nerve ligation (CCI model of neuropathic pain), mechanical hyperalgesia failed to develop by 14 days post-injury [58].

2. Targeting the peripheral endocannabinoid system in chronic pain

A major limitation to the systemic use of cannabinoid agonists as treatment for chronic pain is that activation of cannabinoid receptors in the central nervous system is associated with undesirable side effects, including sedation and catalepsy [59]. Targeting endocannabinoid activity in the peripheral nervous system bypasses these unwanted side effects while still producing analgesia in animal models of inflammatory pain, bone cancer pain, neuropathic pain and sickle cell disease. Continued research into the specific mechanisms of analgesia produced by activation of the endocannabinoid system in the periphery could identify new targets for pain which could serve as stand-alone therapies or be integrated into a multifaceted treatment approach. This chapter will review studies that have investigated the analgesic effects of treatments that target the peripheral endocannabinoid system, whether through direct activation of cannabinoid receptors or through modulation of endocannabinoid metabolism.

2.1. Synthetic cannabinoids in rodent models of pain: inflammation, bone cancer pain, neuropathic pain and sickle cell disease

Local administration of cannabinoid receptor agonists, as opposed to systemic treatment, can produce analgesia without centrally-mediated side effects. Intraplantar administration of the non-selective cannabinoid receptor agonist WIN 55,212-2 attenuated heat and mechanical hyperalgesia in an acute cutaneous heat injury model in rats. This was blocked by a CB1 receptor antagonist, and partially blocked by a CB2 receptor antagonist, suggesting that while both receptor subtypes play a role in anti-nociception during acute pain, the effect was primarily mediated through activation of CB1 receptors [60]. WIN 55,212-2 also decreased mechanical hyperalgesia in the tumor-bearing hind paw in a mouse model of bone cancer pain [61]. The anti-hyperalgesic effect was mediated by both CB1 and CB2 receptors. Importantly, intraplantar administration did not induce catalepsy, which normally occurs when cannabinoid agonists are injected systemically and can confound behavioral measures of nociception [62]. Recordings from the tibial nerve of tumor-bearing mice showed that intraplantar WIN 55,212-2 attenuated sensitization of C-fiber nociceptors as evidenced by a decrease in spontaneous discharge and reduced responses evoked by mechanical stimuli responses evoked by mechanical stimulation, effects which were blocked by both CB1 and CB2 antagonists [63] (**Figure 1**).

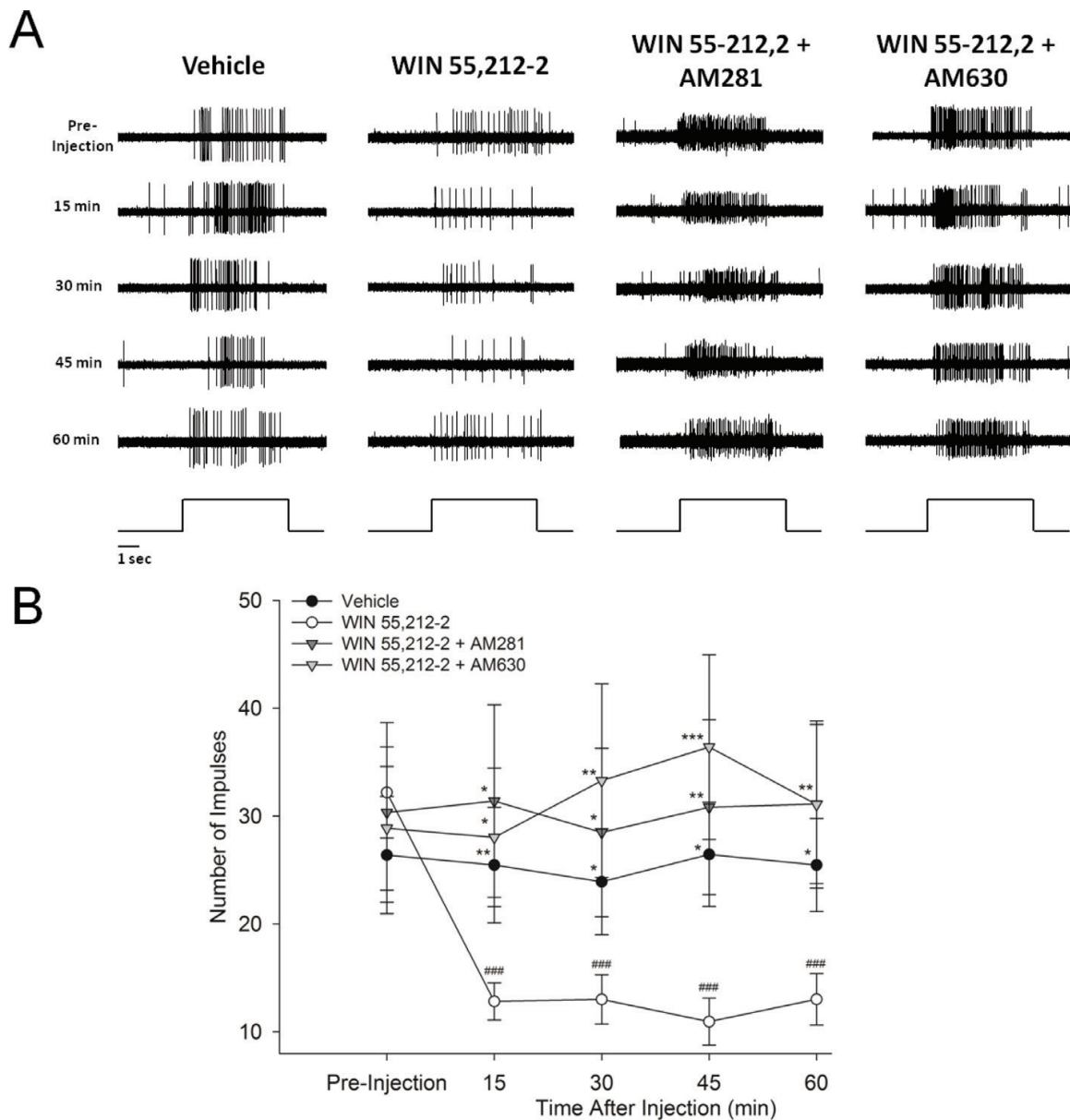


Figure 1. Effect of non-selective cannabinoid receptor agonist WIN 55,212-2 on responses of C-fiber nociceptors evoked by suprathreshold mechanical stimulation. (A) Representative examples of nociceptor responses evoked by 147 mN before injection and at 15, 30, 45 and 60 min after intraplantar administration of vehicle or WIN 55,212-2 alone or preceded by the CB1 receptor antagonist AM281 or CB2 antagonist AM630. The time of application of the stimulus is shown at the bottom of each column. (B) Mean (\pm SEM) number of evoked impulses before and at 15, 30, 45 and 60 min after intraplantar administration of vehicle, WIN 55,212-2, WIN 55,212-2 + AM281, and WIN 55,212-2 + AM630. Evoked responses were not changed following injection of vehicle but decreased following WIN 55,212-2. This was blocked by pretreatment with AM281 or AM630. * $p < .05$, ** $p \leq .01$, *** $p < .001$ vs. WIN 55,212-2; ### $p < .001$ vs. pre-injection value (from Uhelski et al. [63]).

In a model of inflammatory pain, intraplantar administration of the non-selective cannabinoid receptor agonist CP 55,940 attenuated CFA-induced hyperalgesia in mice expressing human sickle hemoglobin (BERK and hBERK1) as well as controls expressing normal human hemoglobin (HbA-BERK) [64].

The analgesic effect of intraplantar WIN 55,212-2 showed more variability in models of neuropathic pain. In a sciatic nerve ligation model, only the highest dose tested (250 μ g) produced an anti-hyperalgesic effect, but the injection altered withdrawal latencies to heat and mechanical response thresholds in both the treated and non-treated hind paw, suggesting that the drug effect was not limited to the periphery [65]. This effect was also seen in rats with partial sciatic nerve ligation (Seltzer model of neuropathic pain); however, the effect was blocked by the intraplantar administration of a CB1 antagonist but not when that same antagonist was administered by the intrathecal route [26], indicating that the ability of WIN 55,212-2 to produce anti-nociception in the contralateral paw is not necessarily mediated by activation of CB1 receptors in the central nervous system. In a rat model of chemotherapy-induced peripheral neuropathy produced by paclitaxel treatment, intraplantar administration of WIN 55,212-2 had no effect on mechanical or heat hyperalgesia, whereas systemic treatment produced anti-nociception [65]. In contrast, intraplantar administration of WIN 55,212-2 attenuated mechanical allodynia associated with streptozotocin-induced diabetic neuropathy [31]. A non-selective cannabinoid receptor agonist naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone, a novel compound which does not appear to cross the blood–brain barrier, reduced mechanical hyperalgesia in a rats with partial sciatic nerve ligation when administered orally [66], indicating that peripherally-restricted activation of cannabinoid receptors can produce adequate analgesia with an oral dosing regimen.

Receptor-selective synthetic cannabinoids also produce analgesic effects. In rats given an intraplantar injection of CFA, arachidonyl-2'-chloroethylamide (ACEA) and (R)-(+)-methanandamide (methAEA), stable mimics of AEA that preferentially bind CB1 receptors, reduced mechanical hyperalgesia and decreased evoked responses in A δ -fiber nociceptors. The reduction in mechanical hyperalgesia was blocked by a CB1 receptor antagonist, but not by a CB2 antagonist. Notably, neither drug had any effect on mechanical withdrawal thresholds or paw withdrawal frequency in naïve rats, and no changes were seen in evoked responses of A δ -fiber nociceptors isolated from nerves innervating normal, non-inflamed paws [67]. The CB1 receptor agonist arachidonylcyclopropylamide (ACPA) attenuated hyperalgesia in a mouse model of bone cancer pain [68].

Intraplantar administration of AM1241, which preferentially binds to the CB2 receptor, reduced withdrawal responses to noxious heat in naïve rats, and no central side effects were observed when this compound was administered systemically [69]. Intraplantar administration of AM1241 reduced capsaicin-evoked nocifensive behaviors and hyperalgesia [70], reduced hyperalgesia and edema in carrageenan-induced inflammation [71], and reduced hyperalgesia in a mouse model of bone cancer pain [68].

The endocannabinoids AEA and 2-AG have also been assessed for their peripheral anti-nociceptive properties. Intraplantar administration of AEA prevented the development of CFA-induced hyperalgesia and inflammation, while systemic administration of AEA had no effect [19]. This indicates that in order for AEA to inhibit the inflammatory pain that follows CFA injection, high levels of the drug must be present at the site of injury, which is difficult to achieve under normal conditions given that AEA has a short half-life due to rapid degradation

by enzymes. Intraplantar AEA also inhibited capsaicin-induced edema and reduced formalin-induced nociceptive behaviors via CB1 receptor activation [15, 19, 72]. Intraplantar AEA was far more effective at inhibiting formalin-induced behaviors than intravenous AEA [15]. Formalin-evoked behaviors were also inhibited by intraplantar administration of 2-AG, an effect blocked by a CB2 receptor antagonist but not a CB1 antagonist [73]. In rats with inflammation produced by carrageenan administration to the hind paw, evoked responses of nociceptive spinal dorsal horn neurons were reduced following intraplantar administration of AEA [74]. The reduction in evoked activity was blocked by a CB2 antagonist, but not a CB1 antagonist. Intraplantar administration of AEA did not produce any changes in evoked responses of spinal neurons in control rats. Intraplantar AEA decreased hyperalgesia in the tumor-bearing paw in a mouse model of bone cancer pain, and this was blocked by a CB1 receptor antagonist [75]. Intraplantar 2-AG also decreased hyperalgesia in the tumor-bearing paw and the anti-hyperalgesia was mediated by CB2 receptors [76]. Intraplantar AEA has also been shown to decrease hyperalgesia following cisplatin treatment [77]. The mechanism of anti-nociception produced by AEA is complex, and the subtype of cannabinoid receptors involved in its effect seems to differ under acute and chronic pain states. AEA has strong analgesic effects when applied to the site of inflammation or neuropathic pain; however, it should be noted that elevated levels of AEA can also increase excitability of nociceptors through activation of TRPV1 receptors that induces Ca^{2+} influx. This effect was shown in cultured dorsal root ganglion (DRG) neurons sensitive to heat stimulation [78]. It should also be noted that endocannabinoid interactions with ion channels and other binding sites separate from cannabinoid receptors can also produce changes in neuronal function.

In addition to direct cannabinoid receptor agonists, there are drugs which modify endocannabinoid metabolism and thereby alter levels of endocannabinoids. For example, compounds that inhibit enzymes that break down endocannabinoids increase the amount of endocannabinoids available for binding to cannabinoid receptors. URB597 ((3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl)-cyclohexylcarbamate) targets fatty acid amide hydrolase (FAAH), an enzyme which breaks down AEA. Intraplantar administration of URB597 decreased hyperalgesia and C-fiber nociceptor sensitization in chemotherapy-induced peripheral neuropathy following cisplatin treatment, effects which were blocked by a CB1 receptor antagonist but not a CB2 antagonist. Biochemical analysis of skin showed that URB597 increased local levels of AEA without altering the levels of other endocannabinoids [79], indicating that increased activation of CB1 receptors by AEA was the source of decreased nociceptor excitability and analgesia. Intraplantar URB597 also decreased hyperalgesia and C-fiber nociceptor sensitization in a transgenic mouse model of sickle cell disease (SCD, HbSS-BERK). These effects were also blocked by a CB1 receptor antagonist but not by a CB2 receptor antagonist (**Figure 2**). Importantly, intraplantar administration of URB597 still had an anti-hyperalgesic effect in sickle mice with CB2 receptors knocked out (HbSS-BERK-CBR2^{-/-}, [80], confirming mediation by CB1 receptors.

Systemic application of URB937 (N-cyclohexyl-carbamic acid, 3'-(aminocarbonyl)-6-hydroxy [1,1'-biphenyl]-3-yl ester), a FAAH inhibitor that is restricted to the periphery and cannot cross the blood-brain barrier, produced analgesic effects in sciatic nerve ligation (Bennett model of

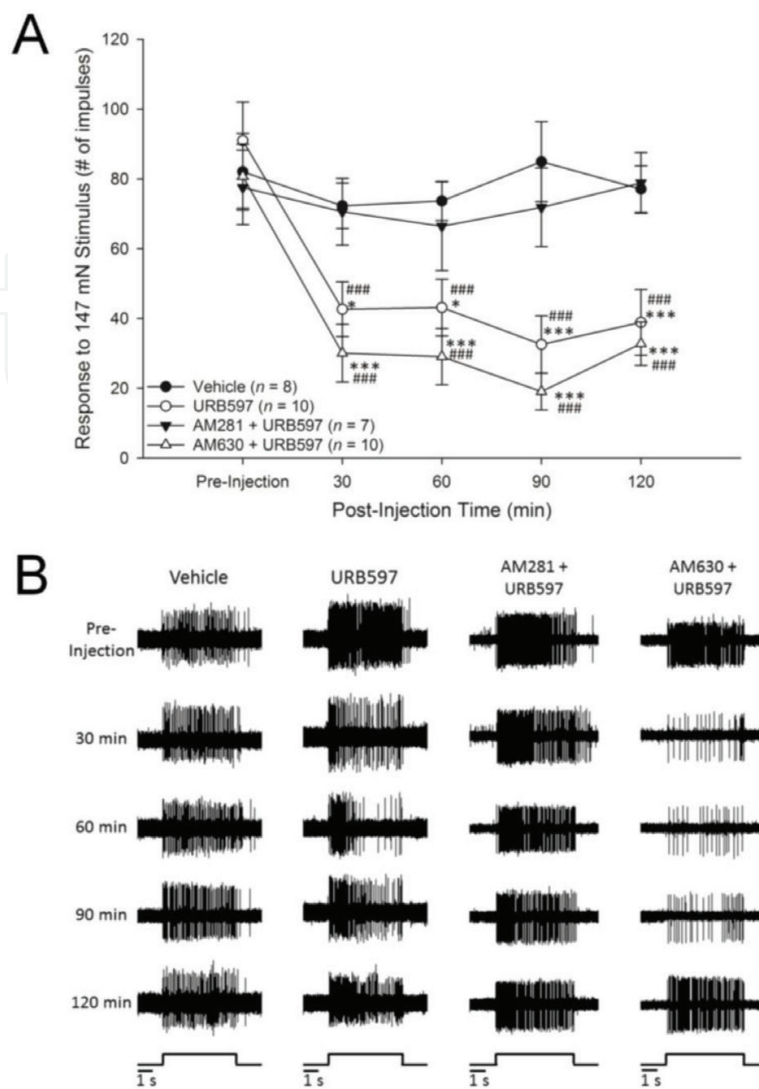


Figure 2. (A) Intraplantar administration of the FAAH inhibitor URB597 decreased evoked responses in C-fiber nociceptors isolated from HbSS-BERK sickle mice. Data show the mean (\pm SEM) number of impulses evoked by 147 mN before and at 30, 60, 90 and 120 min after various drug treatments. The number of evoked impulses was reduced following intraplantar administration of URB597 at 30, 60, 90, and 120 min post-injection, and this effect was blocked by the CB1 receptor antagonist AM281, but not CB2 receptor antagonist AM630. * $p < .05$, ** $p < .005$, *** $p \leq .001$ vs. the vehicle-treated group. ### $p < .001$ indicates significant differences from pre-injection value. (B) Representative examples of responses of individual C-fibers evoked by 147 mN for 5 s before (pre-injection) and at 30, 60, 90 and 120 min after intraplantar injection of vehicle, URB597, URB597 + AM281, and URB597 + AM630. The time of mechanical stimulation is illustrated at the bottom of each column. Reproduced from Uhelski et al. [80].

neuropathic pain) and carrageenan-induced inflammation in the affected hind paw, but did not alter responses on the non-affected hind paw [81, 82]. In cisplatin-treated mice, URB597 delayed and decreased the hyperalgesic effect of cisplatin [77]. Unfortunately, sustained pharmacological inhibition of FAAH results in endocannabinoid catabolism by alternative pathways, which are not dependent upon FAAH [83], thus limiting their clinical effectiveness. FAAH knockout mice have elevated levels of N-acylethanolamines and N-acyl taurines, show reduced responses to noxious stimuli, and are hypersensitive to AEA [84].

Monoacylglycerol lipase (MAGL) is an enzyme that breaks down 2-AG. Inhibition of MAGL produces analgesia under inflammatory conditions. Intraplantar administration of MAGL-inhibitor URB602 (N-[1,1'-Biphenyl]-3-yl-carbamic acid, cyclohexyl ester) attenuated formalin-evoked nociceptive behaviors [73]. Combining URB602 with 2-AG enhanced the anti-nociceptive effects of each [73]. The effect of URB602 was blocked by both CB1 and CB2 antagonists, whereas the effects of 2-AG were only blocked by a CB2 antagonist, suggesting that the URB602 does not behave as selective and/or potent inhibitor of MAGL [85] and that its effects are not dependent on only 2-AG, but may involve the inhibition of FAAH as well. In a mouse model of bone cancer pain, JZL184, a selective MAGL inhibitor, attenuated hyperalgesia in the tumor-bearing hind paw [76]. JZL184 elevates levels of 2-AG but not AEA following acute systemic administration, and the anti-hyperalgesic effect was shown to be dependent on CB2 (but not CB1) receptors. In contrast, intraplantar injection of JZL184 in cisplatin-treated mice decreased hyperalgesia by inhibiting both MAGL and FAAH and normalizing 2-AG and AEA levels in the plantar skin and DRG [86].

2.2. Mechanisms underlying peripheral effects of endocannabinoids

There is a large body of evidence demonstrating that activation of cannabinoid receptors in the periphery produces analgesia. This effect appears to be the result of decreased nociceptor excitability, and there are several mechanisms that could contribute to this effect. These include direct activation of cannabinoid receptors that are expressed by nociceptors as well as activation of cannabinoid receptors expressed in the surrounding non-neuronal tissue that indirectly modulate neuronal excitability.

Studies of mRNA and protein expression have identified CB1 receptors on nociceptive neurons, and selectively knocking out CB1 receptors in Na_v1.8-expressing neurons increased sensitivity to noxious heat, enhanced CFA-induced inflammation, and decreased the analgesic effect of WIN 55,212-2 [87–89]. Further, blocking either CB1 or CB2 receptors in the periphery inhibited the anti-nociceptive effect of systemic WIN 55,212-2 to the same degree, suggesting that peripheral cannabinoid receptors are a major site of action for cannabinoid receptor-mediated analgesia [90]. The application of cannabinoid agonist WIN 55,212-2 and CP 55,940 to cultured primary afferent neurons reduced evoked Ca²⁺ influx in intermediate-diameter neurons, but not small-diameter neurons, though immunoreactivity for CB1 was detected in both cell populations [12, 91]. This indicates that reduction of calcium influx is just one of the inhibitory actions that can result from CB1 activation. Activation of CB1 receptors has also been shown to inhibit the release of calcitonin gene-related peptide (CGRP) from the nerve terminals of nociceptive primary afferent fibers in isolated skin from the rat hind paw [19], which could lead to reduced nociceptor excitability.

Expression of cannabinoid receptors can be modified in chronic pain states, which can enhance the effects of cannabinoid agonists. In a mouse model of bone cancer pain, the DRG ipsilateral to the tumor-bearing hind paw showed increased expression of CB1 receptors. Enhanced CB1 receptor expression in the DRG may explain why small-diameter neurons co-cultured with cancer cells were responsive to CB1 receptor agonists (which attenuated evoked calcium influx), while small-diameter DRG neurons in naïve mice were not [75] (**Figure 3**).

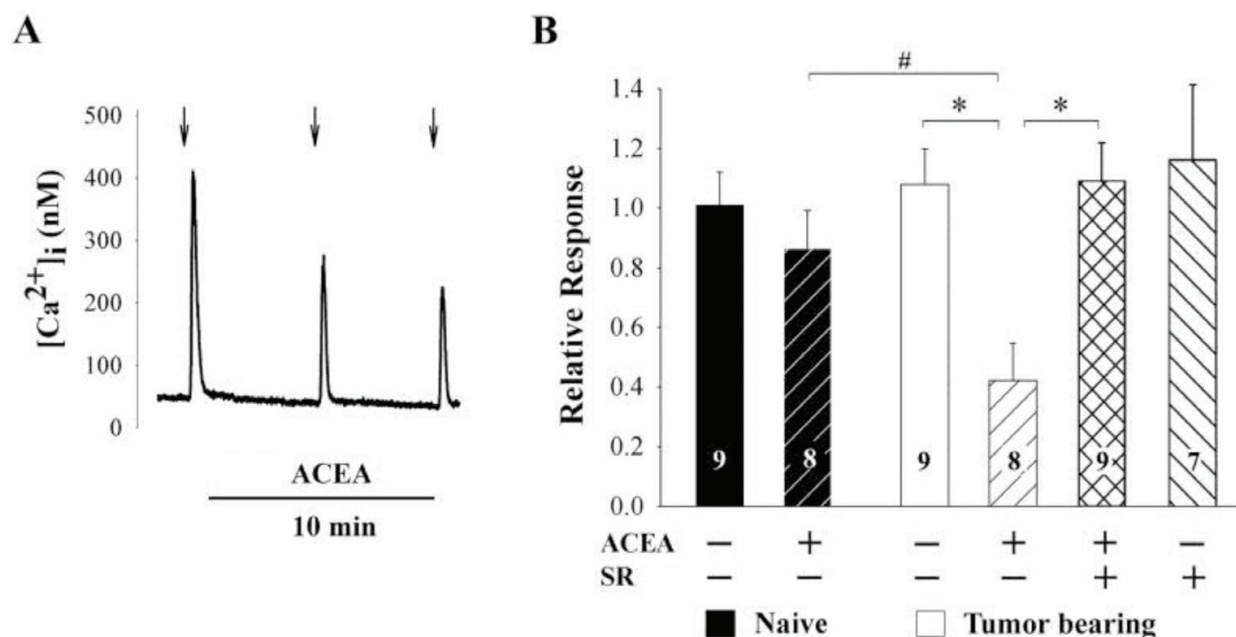


Figure 3. Small DRG neurons (<math><500 \mu\text{m}^2</math>) isolated from DRG L3-L5 of tumor-bearing mice and maintained *in vitro* in control medium for 20–28 h exhibited a change in sensitivity to the CB1 agonist ACEA. (A) The trace represents cannabinoid agonist inhibition of the Ca^{2+} transient evoked by brief superfusion with KCl (50 mM, 10 s, arrows). ACEA (1 μM) was included in the superfusate following the first test with KCl. (B) ACEA attenuated the Ca^{2+} transient evoked by KCl (50 mM) in neurons isolated from L3-L5 DRGs of tumor-bearing mice, but not in those from naïve mice. Involvement of CB1 receptors in the response to ACEA was confirmed by blocking the inhibitory effect by co-application of the CB1 receptor antagonist SR141716A (SR, 1 μM). Relative response was defined as the amplitude of the response of a neuron to KCl in the presence of ACEA divided by the amplitude of the response in the absence of ACEA. *Significantly different at $p < 0.001$; #significantly different at $p < 0.01$ (one-way ANOVA with Tukey’s multiple comparisons test). Reproduced from Khasabova et al. [75].

CB2 receptor mRNA and protein are increased in the lumbar DRG after spinal nerve ligation (SNL) or CCI (Bennett model of neuropathic pain), but not CFA-induced inflammation [92]. The effect appears to be localized to microglia [92, 93], though there is some evidence of enhanced neuronal expression after SNL, including increased expression in the nerve proximal to the ligation [94]. In a mouse model of bone cancer pain, tumors showed high levels of CB2 receptor protein levels, and CB2 receptor proteins were also elevated in plantar skin of the tumor-bearing hind paw [76]. Taken together, these results support to the notion that endocannabinoid-mediated inhibition of peripheral nociceptor activity is necessary to prevent exaggerated responses to noxious stimuli and that tonic activation of endocannabinoids aids in suppressing pain, inflammation, and nociceptor sensitization after injury. Further evidence is shown by differences in levels of endocannabinoids in naïve, acute inflammation, and chronic pain conditions. In models of chronic pain from bone cancer and chemotherapy-induced peripheral neuropathy, the level of AEA was decreased in the skin of the plantar hind paw due to increased FAAH mRNA expression and AEA uptake in DRG neurons ipsilateral to a tumor-bearing hind paw [75, 95]. In cisplatin-treated mice, expression of 2-AG and AEA are both decreased in the plantar skin and DRG [86].

3. Conclusions

Concerns about the safety of commonly used analgesic drugs have hindered the treatment of patients with chronic pain. Continued exploration of mechanisms underlying nociceptive processing under naïve, acute and chronic pain states has helped identify specific targets for the development of new treatment approaches that could solve some of the problems associated with chronic use of opiates and NSAIDs. This includes the use of drugs which target the endocannabinoid system. Early investigations identified problems with the systemic use of compounds derived from the cannabis plant, including sedation, mood alterations, and motor effects, a direct consequence of binding to cannabinoid receptors in the brain. By targeting the peripheral endocannabinoid system, the negative side effects of cannabinoids can be bypassed, providing analgesia without impairment of normal function. Work with animal models has shown that activation of cannabinoid receptors in the periphery can be useful for a wide variety of pain conditions, including inflammation, bone cancer pain, chemotherapy-induced peripheral neuropathy, and sickle cell disease. Analgesia can be achieved through direct receptor activation or through the restoration of endocannabinoid levels, both of which decrease signs of sensitization in peripheral nociceptors. Thus, specific treatments could target known alterations in endocannabinoid levels associated with different chronic pain conditions. Drugs targeting the peripheral endocannabinoid system could be used as effective analgesics or in combination with currently available therapies to maximize pain relief while minimizing harmful side effects.

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Conflict of interest

The authors have no conflicts of interest to declare.

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