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Cannabinoid CB_2 Receptor-Mediated Anti-nociception in Models of Acute and Chronic Pain

Maulik D. Jhaveri · Devi R. Sagar · Steven J. R. Elmes · David A. Kendall · Victoria Chapman

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Abstract The endocannabinoid system consists of cannabinoid CB_1 and CB_2 receptors, endogenous ligands and their synthesising/metabolising enzymes. Cannabinoid receptors are present at key sites involved in the relay and modulation of nociceptive information. The analgesic effects of cannabinoids have been well documented. The usefulness of nonselective cannabinoid agonists can, however, be limited by psychoactive side effects associated with activation of CB_1 receptors. Following the recent evidence for CB_2 receptors existing in the nervous system and reports of their up-regulation in chronic pain states and neurodegenerative diseases, much research is now aimed at shedding light on the role of the CB_2 receptor in human disease. Recent studies have demonstrated anti-nociceptive effects of selective CB_2 receptor agonists in animal models of pain in the absence of CNS side effects. This review focuses on the analgesic potential of CB_2 receptor agonists for inflammatory, post-operative and neuropathic pain states and discusses their possible sites and mechanisms of action.

Keyword CB_2 receptor · Cannabinoid · Nociception · Neuropathic pain · Inflammatory pain · Postoperative pain · Preclinical · Electrophysiology · Weight bearing

Abbreviations

SNL	spinal nerve ligation
CCI	chronic constriction injury
CB_1	cannabinoid CB_1 receptor
CB_2	cannabinoid CB_2 receptor
DRG	dorsal root ganglion
PEA	palmitoyl ethanolamide

Jhaveri and Sagar joint first author.

M. D. Jhaveri (✉) · D. R. Sagar · S. J. R. Elmes · D. A. Kendall · V. Chapman
Institute of Neuroscience, School of Biomedical Sciences, University of Nottingham,
Nottingham NG7 2UH, UK
e-mail: maulik.jhaveri@nottingham.ac.uk

Introduction

Preparations of the hemp plant (*Cannabis sativa*) have been used for the treatment of pain for more than 4,000 years. However, there has been a reluctance to use cannabis-based products in modern medicine, and renewed interest in their development only arose in the 1960s when one of the most active components of cannabis extract, Δ^9-tetrahydrocannabinol (Δ^9-THC), was isolated and partially synthesised [1]. Since then, two cannabinoid receptors, CB_1 and CB_2, have been cloned and pharmacologically characterised [2–7] and endogenous ligands for these receptors identified. Both CB_1 and CB_2 receptors are negatively coupled to adenylyl cyclase through $G_{i/o}$ proteins and are positively coupled to mitogen-activated protein (MAP) kinases (for review see [8]). Over the past few years, several reviews have been published on cannabinoid receptors and the mechanisms of action of cannabinoid ligands, which underlie their analgesic effects [9–17]. The present review will focus on cannabinoid CB_2 receptors and their role in modulating nociceptive processing, which suggests they may have therapeutic potential as analgesics.

Cannabinoid Receptor Distribution

CB_1 receptors are present in remarkably high density mainly on neurones of the central, peripheral and autonomic nervous systems [18–22]. There are more moderate

levels of CB_1 receptors in the testis, heart, lungs, uterus, ovary, bone marrow, thymus, tonsils and immune cells [4, 23]. CB_2 receptors are mainly present in the immune system at an expression level much higher than that of CB_1 [23]. Until recently, it had been thought that CB_2 were not constitutively present in the brain; however, evidence is coming to light for the presence of CB_2 receptors on brain microglial cells [24–29] and on neurones in several brain regions, including the cerebellum [30], brainstem [31], periaqueductal grey (PAG), thalamus, striatum, cortex, amygdala and hippocampus [32]. CB_2 receptors are also present in skin [33–36] and the spinal cord [35–37]. The presence of CB_2 receptors on dorsal root ganglion (DRG) neurones is slightly more contentious with evidence existing for both the presence [37–39] and absence [22, 40] of these receptors. The basis for these differences is unclear, but they may arise as a result of culture conditions influencing receptor expression or the poor specificity of antibodies employed. It has been hypothesised that after nerve injury, CB_2 receptors are synthesised in DRG cell bodies and rapidly transported out to nerve terminals in vivo [38] similar to the process previously demonstrated for CB_1 receptors [22]. The presence of CB_2 receptor protein in isolated DRGs from nerve-injured rats and the accumulation of CB_2 receptor-immunoreactivity in sciatic nerve axons proximal, but not distal, to the nerve ligation supports the expression of CB_2 receptors by sensory neurones [38].

Antinociceptive Effects of CB_2 Receptor Agonists in Models of Acute Pain

The efficacy of cannabinoids as analgesic agents has been widely demonstrated [10, 17], although the use of many of these compounds has been limited by psychoactive side effects that accompany their anti-nociceptive actions. In addition, cannabinoids can produce motor deficits, which may confound analgesic effects observed in animal behavioural studies of acute pain. Given that the unwanted side-effects of cannabinoids arise as a result of activation of centrally located CB_1 receptors, recent studies have focused on the analgesic effects of CB_2 receptor agonists. Indeed, CB_2 receptor activation has been shown to be devoid of CNS-mediated side effects, such as, catalepsy, hypothermia and reduced locomotion that result from CB_1 receptor activation [41, 42].

The anti-nociceptive potential of CB_2 receptor agonists was first indicated by studies using the endogenous fatty acid derivative palmitoylethanolamide (PEA). PEA was demonstrated to have marked anti-inflammatory [43, 44] as well as anti-nociceptive effects [45–48] when administered in vivo. Although PEA is not an agonist at CB_1 or CB_2 receptors, its anti-nociceptive effects were blocked by the CB_2 receptor selective antagonist SR144528, which implicates an indirect role of CB_2 receptors in the effects of PEA, or the involvement of a CB_2-like receptor.

A number of selective CB_2 receptor agonists have been shown to be anti-nociceptive in models of acute pain (Table 1). Malan et al. [42] demonstrated that the CB_2 receptor selective agonist AM1241 increased hind-paw thermal withdrawal latency; an effect which was blocked by the CB_2 receptor selective antagonist AM630, but not by the CB_1 receptor selective antagonist AM251, indicating mediation by CB_2 receptors. This was further confirmed in a later study, which demonstrated that the anti-nociceptive effects of AM1241 were absent in CB_2 receptor-null mice [49]. Importantly, Malan and coworkers have demonstrated that AM1241-mediated anti-nociception was devoid of CNS side effects, as indicated by the lack of effects on immobility/catalepsy, locomotor activity and hypothermia [42]. Similarly, another CB_2 receptor selective agonist, GW405833, has been shown to be anti-nociceptive in the

Table 1 Antinociceptive effects of CB_2 receptor agonists in animal models of acute and post-operative pain in vivo

Noxious stimulus	Species	Agonist	Effect	Route of administration	References
Thermal stimulus to hindpaw	Rat	AM1241	Increased paw withdrawal latency	i.pl., i.p.	[33, 42]
	Rat	GW405833	Increased paw withdrawal latency	i.p.	[50]
Tail Flick (thermal)	Rat	GW405833	Increased latency of tail flick	i.p.	[50]
	Mouse	AM1241	Increased latency of tail flick	i.p.	[49]
Incision to hindpaw	Rat	GW405833	Reduction in mechanical hypersensitivity	i.p.	[50, 65]
	Rat	AM1241	Attenuation of incision-induced allodynia of hindpaw	i.p.	[65]
	Rat	HU308	Attenuation of incision-induced allodynia of hindpaw	i.p.	[65]
Mechanical stimulus to hindpaw	Rat	JWH133	Inhibition of mechanically evoked responses of spinal dorsal horn neurones	i.pl.	[52]

i.p., intraperitioneal administration; *i.pl.*, intraplantar administration

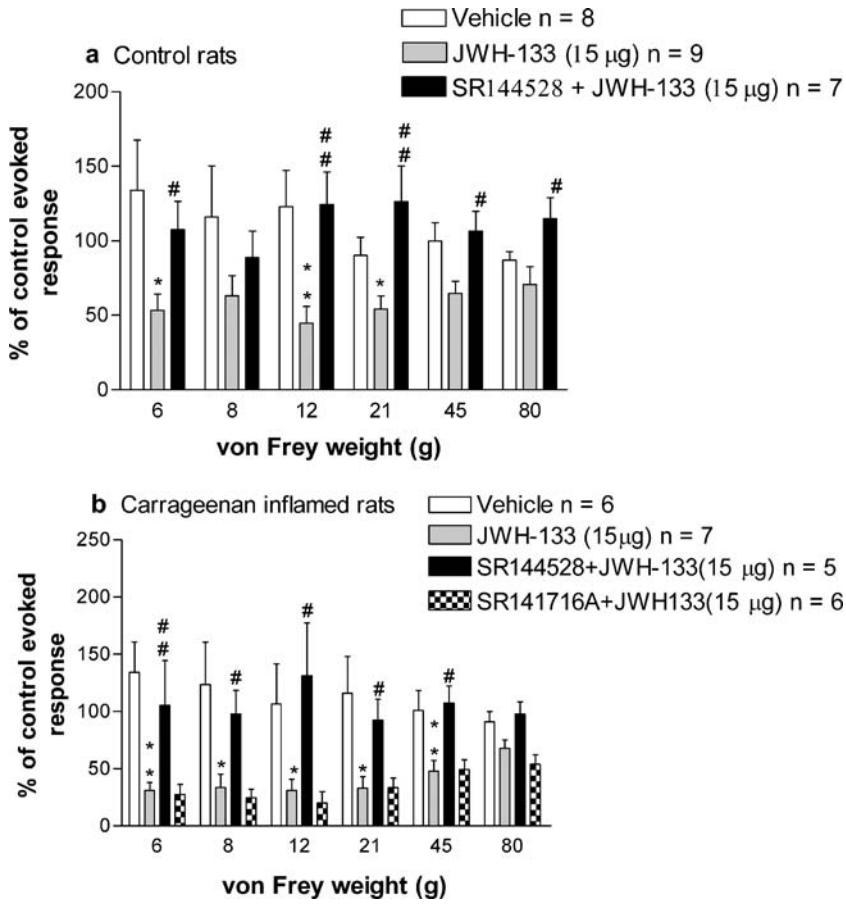

Fig. 1 Mean maximal effects of intraplantar injection of JWH-133 on mechanically evoked responses of WDR spinal neurones in; **a** non-inflamed rats and **b** rats with established carrageenan-induced hindpaw inflammation. Saline/carrageenan (2 mg in 100 μl) was injected intraplantar and mechanically evoked responses recorded every 10 min. Intraplantar injection of selective CB_2 agonist, JWH-133 (15 μg/50 μl), 180 min post-saline/carrageenan, reduced mechanically evoked responses. Intraplantar injection of CB_2 antagonist SR144528 (10 μg in 50 μl) but not CB_1 antagonist SR141716A (10 μg in 50 μl), 30 min prior to JWH-133, significantly inhibited the effects of JWH-133. Statistical comparisons between groups were performed with non-parametric Mann-Whitney test. *Asterisk*, $P<0.05$; *double asterisk*, $P<0.01$ vs vehicle control; *number sign*, $P<0.05$; *double number sign* $P<0.01$ vs JWH-133 (15 μg/50 μl). Data are expressed as mean percentage of control-evoked responses+SEM (n=neurones/group). Figure modified from [52]

tail flick and hotplate assays of acute nociception, although some effects were accompanied by catalepsy and motor-incoordination in the rotorod test [50].

Electrophysiological studies in our group have demonstrated that peripheral administration of the CB_2 receptor specific agonist JWH-133 [51] produces anti-nociceptive effects in naïve rats [52]. In this study, intraplantar injection of JWH-133 (15 μg in 50 μl) significantly inhibited both innocuous and noxious mechanically evoked responses of dorsal horn neurones in anesthetised rats in vivo (Fig. 1). Inhibitory effects of JWH-133 were blocked by the CB_2 receptor antagonist SR144528 [53], but not the CB_1 receptor selective antagonist SR141716A [52, 54].

CB_2 Receptor Ligands are Anti-nociceptive in Models of Inflammatory Pain and Post-operative Pain

Inflammatory pain states are a major clinical problem, and the presence of CB_2 receptors on immune cells and peripheral nerves means that there is a strong likelihood that they will produce anti-nociceptive effects in models of inflammatory pain. Systemic administration of one of the first selective CB_2 receptor agonists, HU308, reduced inflammatory pain in the mouse formalin test and arachidonic acid-induced inflammatory ear oedema [41]. Following this early report, the anti-nociceptive effects of systemic administration of AM1241, JWH015 and GW405833 have been reported in the carrageenan, Freund's complete adjuvant and colonic distention models of inflammatory pain [50, 55–58]. Similar to the effects of HU308, systemic administration of JWH133 and GW405833 also reduced inflammatory oedema due to carrageenan or the histamine releasing compound 48/80 [57, 59]. These effects of systemically administered CB_2 agonists may arise from local peripheral, spinal or possibly supraspinal sites of action (Table 2).

Studies using local administration of CB_2 receptor agonists have investigated the location of sites mediating these analgesic effects. Intraplantar injection of the CB_2 receptor agonist AM1241 reduced carrageenan-induced

Table 2 Antinociceptive effects of CB_2 receptor agonists in animal models of inflammatory and neuropathic pain in vivo

Noxious stimulus	Species	Agonist	Effect	Route of administration	References
Formalin into hindpaw	Mouse	HU-380	Inhibition of late phase pain behaviour	i.p.	[41]
Carrageenan inflammation of hind-paw	Rat	JWH-133	Inhibition of mechanically evoked responses of spinal dorsal horn neurones	i.pl.	[52]
			Attenuation of decreases in hindpaw weight bearing	i.p.	[85]
		AM1241	Reversal of thermal hyperalgesia	i.pl.	[60]
			Suppression of thermal and mechanical hyperalgesia	i.p., i.pl.	[55]
	Rat	GW405833	Inhibition of decrease in weight-bearing	i.p.	[57]
Freund's adjuvant into hindpaw	Rat	GW405833	Reduction in mechanical hyperalgesia	i.p.	[50, 56]
	Mouse	GW405833	Reduction in mechanical hyperalgesia	i.p.	[50]
Spinal nerve ligation (L5 and L6)	Rat	JWH-133	Inhibition of mechanically evoked responses of spinal dorsal horn neurones	i.pl., spinal	[52, 74]
	Rat	AM1241	Inhibited tactile allodynia and thermal hypersensitivity	i.p.	[72]
	Mouse	AM1241	Increased tactile withdrawal thresholds to pre-ligation values	i.p.	[72]
Partial sciatic nerve ligation	Rat	GW405833	Reduction in mechanical hyperalgesia	i.p.	[50]
	Mouse	GW405833	Reduction in mechanical hyperalgesia	i.p.	[56]

i.p., intraperitioneal administration; *i.pl.*, intraplantar administration

thermal hypersensitivity, an effect that was blocked by the selective CB_2 antagonist AM630 [60]. Electrophysiological studies by our group using intraplantar administration of drugs showed significant inhibitory effects of the CB_2 receptor agonist JWH133 (15 μg in 50 μl) on mechanically evoked responses of dorsal horn neurones in rats with an established carrageenan-induced inflammation (Fig. 1). This effect of JWH133 was blocked by the CB_2 receptor antagonist SR144528 but not by SR141716A (Fig. 1) [52]. Not only did JWH-133 attenuate evoked responses of spinal neurones, but it also significantly attenuated carrageenan-induced increases in receptive field size of dorsal horn neurones (Fig. 2) [52]. This reduction in evoked responses and receptive field size by JWH133 in carrageenan-inflamed rats appears to be translated into overt behavioural anti-nociception. In behavioural experiments, the ability of JWH133 (3 mg/kg ip) to prevent the development of changes in weight bearing on the carrageenan-inflamed hindpaw was studied. JWH-133 was shown to attenuate mechanical hypersensitivity in carrageenan-inflamed rats (Fig. 3), indicating that activation of CB_2 receptors attenuates inflammation-induced hyperalgesia. Endogenous cannabinoids such as anandamide and 2-arachidonoyl glycerol have also been shown to produce CB_2 receptor-mediated anti-

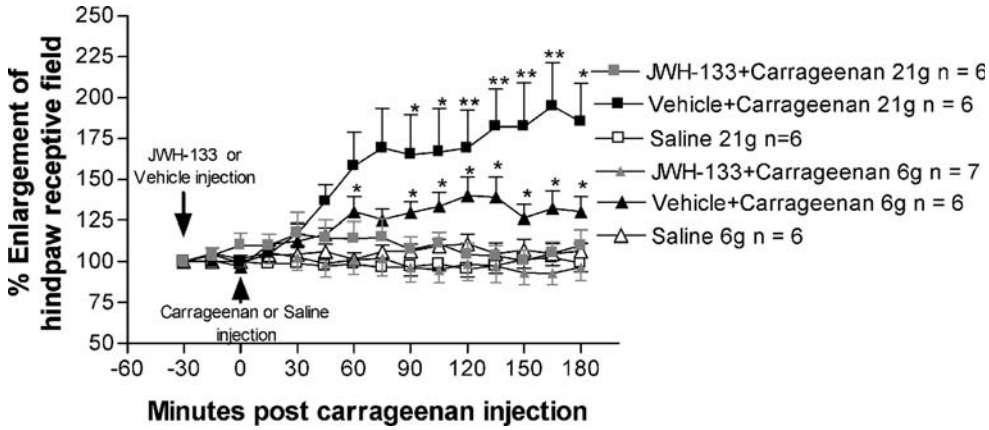

Fig. 2 Effects of intraplantar injection of JWH-133 (15 μg in 50 μl) or vehicle on carrageenan-induced expansion of peripheral receptive fields of WDR neurones as determined with innocuous (6 g) and noxious (21 g) mechanical stimuli. Saline or carrageenan (2 mg in 100 μl) was injected intraplantar and receptive field size measured every 15 min. Intraplantar injection of selective CB_2 agonist, JWH-133 (15 μg/50 μl), 180 min post-carrageenan, inhibited the carrageenan-induced enlargement in receptive field. Statistical comparisons between groups were performed with Mann-Whitney non-parametric test; *asterisk*, $P< 0.05$; *double asterisk*, $P<0.01$ vs JWH-133+ carrageenan. Data are expressed as means±SEM (n=neurones/group). Figure modified from [52]

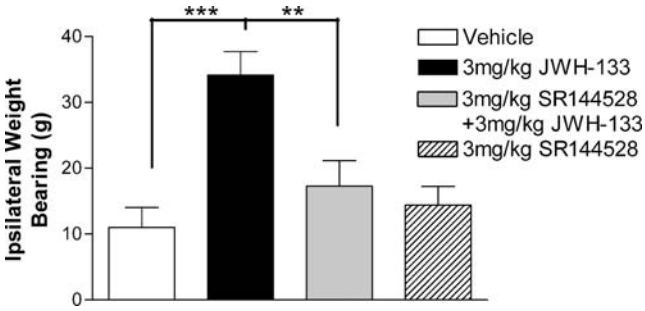

Fig. 3 Effects of post-administration of vehicle, JWH-133, SR144528 +JWH-133 and SR144528 on maintenance of carrageenan-induced decrease in weight bearing on the left hind-paw. Intraperitoneal injection of selective CB_2 agonist, JWH-133 (3 mg/kg), 3 h post-carrageenan injection, inhibited carrageenan-induced reduction in weight bearing on the ipsilateral hindpaw. Intraperitoneal injection of selective CB_2 antagonist, SR144528 (3 mg/kg), 30 min before JWH-133, inhibited the effects of JWH-133. Statistical analysis compared pairs of test groups using Tukey's post hoc test, *df*=27; *double asterisk*, $P<0.01$; *triple asterisk*, $P<0.001$. Data are expressed as mean ipsilateral weight bearing±SEM ($n=7$ for each group). Figure modified from [85]

nociception in models of pain. Anandamide attenuated bladder inflammation-induced referred hindpaw hyperalgesia and bladder hyper-reflexia in rats via CB_1 and CB_2 receptor mechanisms [47, 48]. In another report, anandamide was reported to reduce mechanically evoked responses of dorsal horn neurones in rats with hindpaw inflammation via CB_2 receptors [61]. Similarly, 2-arachidonoyl glycerol reduces the late phase of formalin-evoked nociceptive responses via CB_2 receptors [62]. A detailed discussion on the role of endocannabinoids in nociception is beyond the scope of this article and is reviewed elsewhere [13, 63].

The ability of CB_2 receptor agonists to modulate post-operative pain has been investigated. As yet, the mechanisms underlying this type of pain are not fully understood; however, it is thought that it comprises both neuropathic and inflammatory elements. A rat model of post-operative pain involving incisional injury produces robust mechanical allodynia 2 h post-incision, a time-course similar to that experienced by humans [64]. The CB_2 receptor agonists AM1241, HU308 and GW405833 all produce dose-dependant reductions in mechanical hyperalgesia and tactile allodynia in this model [50, 65].

CB_2 Receptors: An Important Analgesic Target for Neuropathic Pain States

Although CB_2 receptors have recently been reported to be present in the brain, their functional role in normal physiological conditions is unclear. Nevertheless, under pathological conditions such as Alzhemier's disease [66, 67], CB_2 receptors are reported to be up-regulated in the brain and in multiple sclerosis [68, 69] and in neuropathic pain states, CB_2 receptors are up-regulated in the spinal cord [70]. An increase in CB_2 receptor mRNA has been reported in the ipsilateral spinal cord of rats in both the spinal nerve ligation (SNL) and chronic constriction injury models of neuropathic pain. By contrast, spinal levels of CB_2 receptor were not altered in the carrageenan model of inflammatory pain [70]. The increase in CB_2 mRNA in the ipsilateral spinal cord following SNL injury was subsequently confirmed by another group [37]. Beltramo et al. [37] did, however, report expression of CB_2 mRNA in the spinal cord of sham-operated rats and in the contralateral spinal cord of SNL rats. Studies of CB_2 receptor protein expression, using immunoblot analysis, have also revealed the presence of constitutive CB_2 receptors in the spinal cord and skin of neuropathic mice and rats [35, 36]. Immunohistochemical evidence also supports the pathological expression of CB_2 receptors in the ipsilateral spinal cord and peripheral nerves of SNL rats [38]. Overall, there is mounting evidence for an increase in expression of CB_2 receptors in the hindpaw skin, primary afferents and dorsal horn of spinal cord of neuropathic rats.

Given the up-regulation of CB_2 receptors at key sites involved in pain processing, CB_2 receptor agonists may be effective in clinical neuropathic pain states. Using either a non-selective CB agonist and blocking the effects with a selective CB_2 antagonist [71] or using selective CB_2 agonists such as GW405833 [50, 56] and AM1241 [72], a handful of behavioural studies have reported anti-nociceptive effects of CB_2 receptor activation in models of neuropathic pain (Table 2). By contrast, a recent study using intravenous administration of the non-selective cannabinoid agonist WIN55212-2 has reported a CB_1, but not CB_2-receptor-mediated reduction in thermally evoked responses of dorsal horn wide dynamic range neurones and a reduction in size of receptive field in chronic constriction injury-induced (CCI) neuropathic rats [73].

Electrophysiological studies by our group have shown that both local peripheral and spinal administration of the selective CB_2 receptor agonist JWH133 produces anti-nociceptive effects in neuropathic rats. Intraplantar injection of JWH133 (15 μg in 50 μl) significantly inhibited mechanically evoked neuronal responses in anaesthetised SNL and sham-operated rats (Fig. 4) [52]. These effects of JWH133 on mechanically evoked responses of dorsal horn neurones were attenuated by the selective CB_2 receptor antagonist SR144528 (10 μg in 50 μl) in both sham-operated and SNL rats (Fig. 4).

Studies at the level of the spinal cord revealed marked differences between the effects of the CB_2 receptor agonist in SNL and sham-operated rats [74]. Spinal administration of JWH133 (8–468 ng in 50 μl) significantly reduced mechanically evoked responses of dorsal horn neurones in SNL, but not sham-operated rats. Effects of JWH-133 in

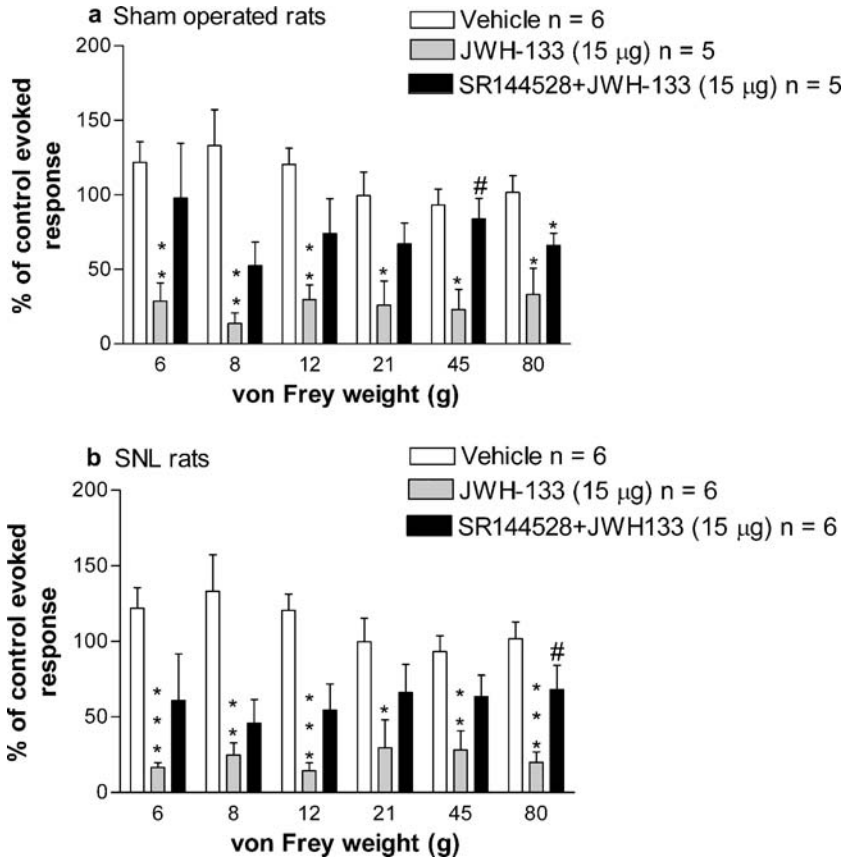

Fig. 4 Mean maximal effects of peripheral JWH-133 on mechanically evoked responses of wide dynamic range dorsal horn neurones in **a** sham-operated and **b** spinal nerve ligated (*SNL*) rats. Intraplantar injection of JWH-133 (15 μg/50 μl) reduced the mechanically evoked responses, an effect partially reversed by SR144528 (10 μg/50 μl), in both sham-operated and SNL rats. Statistical comparisons between treatment groups were performed with non-parametric Mann-Whitney test; *asterisk*, $P<0.05$; *double asterisk*, $P<0.01$; *triple asterisk*, $P<0.001$ vs vehicle; *number sign*, $P<0.05$ vs JWH133. Data are expressed as mean+SEM (n=neurones/group). Figure modified from [52]

SNL rats were blocked by a CB_2 receptor antagonist, but not a CB_1 receptor antagonist (Fig. 5). Overall, our data indicate that, following spinal nerve injury, there is a functional up-regulation of CB_2 receptors in the spinal cord and that activation of these receptors can attenuate evoked responses of spinal neurones. This functional role of spinal CB_2 receptors in neuropathic rats corroborates the report of the spinal expression of CB_2 mRNA in the spinal cord of neuropathic, but not naïve or inflamed rats [70].

Putative Mechanisms Mediating the Analgesic Effects of CB_2 Receptor Agonists

Based on the location of CB_2 receptors and the well-established mechanisms of nociceptive processing, it is likely that the anti-nociceptive effects of CB_2 receptor ligands arise from multiple sites of action (Fig. 6). The mechanism of action of CB_2 receptor agonists administered directly into the skin may be through the activation of CB_2 receptors on keratinocytes, immune cells [44] and primary afferent neurones. Previous studies by Wotherspoon and co-workers have suggested that CB_2 receptors are axonally transported from DRG cell bodies and expressed on nerve terminals [38]. In addition, CB_2 receptor agonists inhibit sensory nerve depolarisation in isolated guinea-pig vagus nerve preparations induced by hypertonic saline, capsaicin and PGE_2 in vitro [75]. Furthermore, there is pharmacological evidence for the presence of CB_2 receptors on peripheral nerve terminals in the vas deferens [76]. Thus, CB_2 receptor agonists may directly inhibit primary afferent neurone depolarisation. In support of this, we have demonstrated inhibitory effects of the CB_2 receptor selective agonist JWH-133 on capsaicin-evoked calcium responses in adult rat DRG neurones in vitro [74]. CB_2 receptors have also been shown to be present on keratinocytes, stimulation of which has been shown to increase local levels of β-endorphin and to produce behavioural anti-nociception in naïve rats [33]. The involvement of β-endorphin in the peripheral effects of the CB_2 agonist AM1241 was confirmed by the absence of anti-nociceptive effects of AM1241 in μ-opioid receptor knockout mice

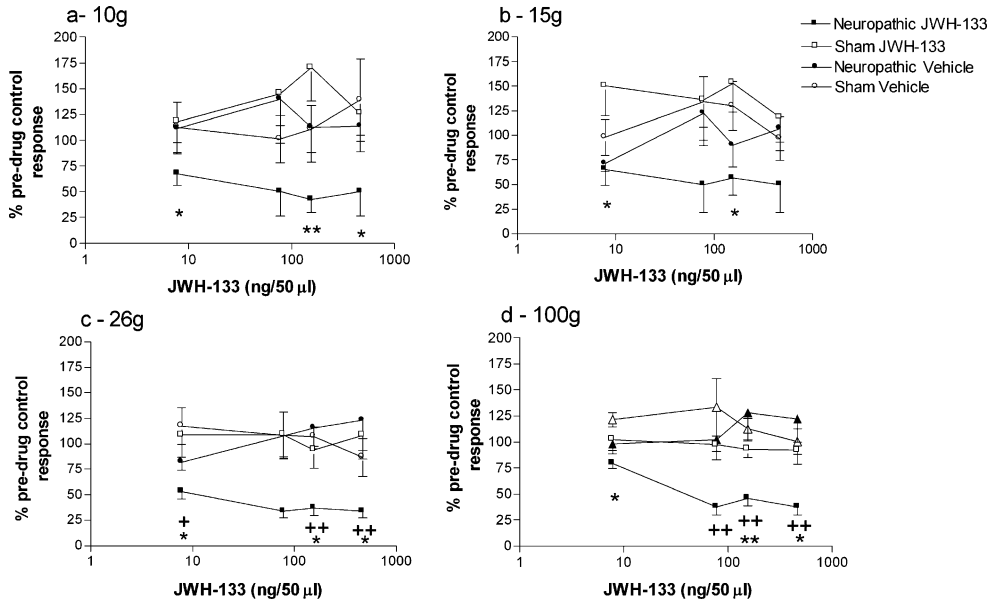

Fig. 5 Mean maximal effects of spinal JWH-133 on mechanically evoked (10–100 g; **a** 10 g, **b** 15 g, **c** 26 g, **d** 100 g) responses of dorsal horn wide dynamic range neurones in neuropathic (SNL) and sham-operated anaesthetised rats in vivo. JWH-133 (8–468 ng/50 µl) reduced mechanically evoked responses in SNL, but not sham-operated rats. Statistical comparison between neuropathic and sham-operated rats was carried out using a Mann-Whitney test where *asterisk*, $P<0.05$; *double asterisk*, $P<0.01$. Statistical comparison between neuropathic and vehicle treated neuropathic rats was carried out using a Mann-Whitney test where *plus sign* denotes $P<0.05$; *double plus sign*, $P<0.01$. Figure modified from [74]

[33]. In addition, AM1241 increased levels of β-endorphin in the skin of naïve rats but not of CB_2 knockout mice [33]. Nevertheless, the mechanisms by which CB_2 receptor activation drives the release of β-endorphin from keratinocytes is unclear.

CB_2 receptor agonists applied directly to the spinal cord reduce nociceptive transmission in neuropathic rats (Figs. 5 and 6b). CB_2 receptor agonists attenuate calcium responses in adult rat DRG neurones from sham-operated and SNL rats [74] and inhibit the release of calcitonin gene related peptide from spinal cord slices of naïve rats [37], indicating an inhibitory effect of CB_2 receptor activation on primary afferent fibres and spinal cord nociceptive transmission. CB_2 receptor immunoreactivity is present in the superficial layers of the dorsal horn and primary afferent fibre terminals of neuropathic but not naïve rats [38], suggesting that CB_2 receptor agonists may indeed act on presynaptic CB_2 receptors to reduce neurotransmitter release in neuropathic rats. CB_2 agonists may also act on CB_2 receptors present on post-synaptic neurones to produce hyperpolarisation and subsequent inhibitory effects in neuropathic rats. Importantly, spinal administration of JWH-133 in sham-operated rats did not alter evoked responses of spinal neurones, indicating that, if present, spinal CB_2 receptors in sham-operated/naive rats are not functional.

Neuropathic pain states are associated with activated immune cells (represented by microglia and astrocytes in the spinal cord); for review, see [77]. Studies of the SNL model of neuropathic pain have shown an early activation of spinal microglia followed by a sustained activation of astrocytes, which contribute to aberrant pain behaviours including mechanical allodynia [78]. Activated glia release proinflammatory cytokines (PIC) including TNF-α, IL-1β, IL-6; all of which contribute to elevated levels of spinal excitability in models of neuropathic pain (see refs in [79]) and enhanced neurotransmitter release from primary afferent fibres [80]. Activation of the cannabinoid receptor system has potent anti-inflammatory effects; endocannabinoids are synthesised by microglia and astrocytes and cannabinoid receptor stimulation modulates cytokine responses in a variety of systems and disease states (see refs in [81]). Up-regulated CB_2 receptors in the spinal cord of neuropathic rats are suggested to be present on glia [82] and, therefore, CB_2 receptors are potentially able to directly modulate the activity of activated glia in the spinal cord of neuropathic rats. Activation of cannabinoid receptors by endocannabinoids and synthetic cannabinoids has anti-inflammatory effects as demonstrated by a reduction in the release of TNFα from cultured microglia [27, 83]. Furthermore, increasing extra-cellular levels of endocannabinoids, by blockade of the anandamide transporter, attenuates the production of proinflammatory mediators by activated astrocytes via both CB_1 and CB_2 receptor activation [84]. Collectively these data suggest that activation of cannabinoid receptors can dampen down proinflammatory responses of microglia and astrocytes, which may contribute to the inhibitory actions of CB_2 receptor agonists in neuropathic rats.

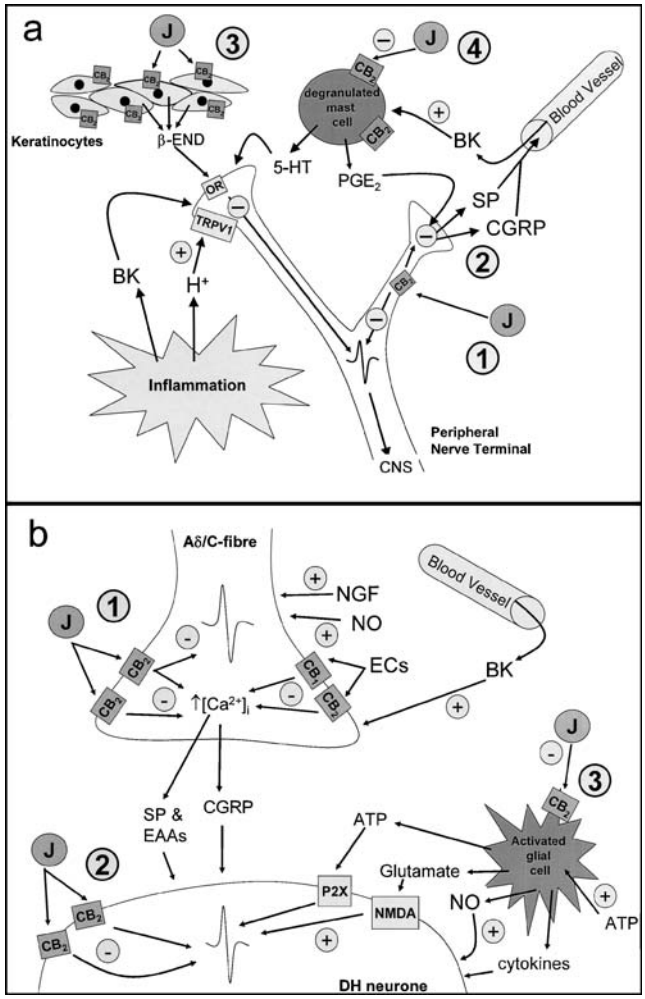

Fig. 6 Possible mechanism of action of CB_2 receptor agonists in the **a** periphery and the **b** spinal cord. **a** In the periphery, JWH-133 (*J*) may exert its inhibitory actions on primary afferent fibres by hyperpolarisation of neurons (*1*); reduction in release of pro-nociceptive neurotransmitters such as calcitonin gene-related peptide (*CGRP*) and substance P (*SP*; *2*); via release of antinociceptive endogenous opioid, β-endorphin (*β-END*) from keratinocytes which can further act on μ-opioid receptors (*OR*) present on nerve terminals (*3*) and; by preventing release of primary afferent fibre sensitizing agents such as serotonin (*5-HT*) and prostaglandin E_2 from immune cells such as mast cells (*4*); *Plus sign*, activation; *minus sign*, inhibition. **b** Following peripheral nerve injury, spinal neurones are sensitized via release of sensitizing agents such as bradykinin (*BK*) adenosine triphosphate (*ATP*), glutamate, nitric oxide (*NO*) nerve growth factor (*NGF*) and cytokines. In the spinal cord of nerve-injured rats, JWH-133 may exert inhibitory effects by acting on up-regulated CB_2 receptors pre-synaptically to reduce intracellular calcium, inhibiting the release of sensitizing factors or by decreasing primary afferent activity and subsequently decreasing the input to dorsal horn (*DH*) neurones (*1*); by action on post-synaptic CB_2 receptors to decrease activity of DH neurones (*2*); or by inhibiting activation of glial cells and release of sensitizing factors (*3*)

In summary, cannabinoid CB_2 receptors are not limited to the immune system, but they are also found in the nervous system where their expression is increased in painful states. Selective activation of CB_2 receptors produces anti-nociception in animal models of pain in the absence of CNS side effects. Therefore, CB_2 receptors provide a potential target for development of new medicines to treat pain.

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