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REVIEW

Cannabinoid CB_2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain

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Cannabinoids suppress behavioural responses to noxious stimulation and suppress nociceptive transmission through activation of CB_1 and CB_2 receptor subtypes. CB_1 receptors are expressed at high levels in the central nervous system (CNS), whereas CB_2 receptors are found predominantly, but not exclusively, outside the CNS. CB_2 receptors are also upregulated in the CNS and dorsal root ganglia by pathological pain states. Here, we review behavioural, neurochemical and electrophysiological data, which identify cannabinoid CB_2 receptors as a therapeutic target for treating pathological pain states with limited centrally, mediated side effects. The development of CB_2-selective agonists (with minimal affinity for CB_1) as well as mutant mice lacking CB_2 receptors has provided pharmacological and genetic tools required to evaluate the effectiveness of CB_2 agonists in suppressing persistent pain states. This review will examine the efficacy of cannabinoid CB_2-selective agonists in suppressing acute, inflammatory and neuropathic nociception following systemic and local routes of administration. Data derived from behavioural, neurochemical and neurophysiological approaches are discussed to better understand the relationship between antinociceptive effects induced by CB_2-selective agonists in behavioural studies and neural mechanisms of pain suppression. Finally, the therapeutic potential and possible limitations of CB_2-based pharmacotherapies for pathological pain states induced by tissue and nerve injury are discussed.
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Abbreviations: CNS, central nervous system; PEA, palmitoylethanolamide

Introduction

The management of chronic and severe pain is the burden of clinicians. Multiple pharmacological agents have been employed to treat diverse pathological pain states including opiates, nonsteroidal anti-inflammatory drugs, anticonvulsants, antidepressants, ketamine and others (Guindon *et al*., 2007). However, adverse side effects constrain therapeutic dosing and limit therapeutic efficacy. Despite improvements in our understanding of pathophysiological mechanisms underlying chronic pain states and the identification of multiple analgesic mechanisms, the clinical need for pharmacotherapies for chronic pain that are effective, nontoxic and devoid of unwanted central side effects remains predominant.

Terminology

Animal models have been developed to experimentally assess pathophysiological mechanisms underlying distinct clinical pain states induced by tissue injury, inflammation, nerve trauma, chemotherapeutic agents and metabolic challenges. These models also permit preclinical evaluation and validation of the therapeutic efficacy of putative analgesics (for review see Dubner and Ren, 1999). Although the mechanisms underlying distinct pathological pain states differ and remain incompletely understood, persistent pain states may share common features. These features include the development of hyperalgesia and/or allodynia and the presence of spontaneous pain. Hyperalgesia is described as an increase in pain evoked by noxious stimuli and also a lowered threshold for pain. Allodynia is defined as an increase in sensitivity to previously non-noxious levels of stimulation. The term hyperalgesia, however, has also been used in the literature to collectively refer to both hyperalgesia and allodynia. This review will describe empirical studies from the literature, which evaluate the utility of exploiting cannabinoid CB_2 receptor mechanisms for suppressing acute, inflammatory and neuropathic pain states.

Historical perspective

Indirect evidence first implicated a role for CB_2 receptor mechanisms in the modulation of persistent pain states.

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Systemic and intraplantar (Calignano *et al*., 1998, 2001) administration of palmitoylethanolamide (PEA), an endogenous fatty acid amide, produces antinociception in the formalin test that is blocked by SR144528, a CB_2 receptor-selective antagonist (Calignano *et al*., 1998). Orally administered PEA also reduced inflammatory hyperalgesia and oedema by inhibiting mast cell degranulation (Mazzari *et al*., 1996) and subsequent release of inflammatory mediators that excite nociceptors. However, PEA does not bind to CB_2 receptors, demonstrating that PEA is not a direct CB_2 receptor agonist (Showalter *et al*., 1996; Griffin *et al*., 2000; De Petrocellis *et al*., 2002; Lo Verme *et al*., 2005).

The subsequent development and evaluation of CB_2-selective agonists such as HU308, AM1241, JWH-133 and GW405833 (L768242) have provided direct support for the hypothesis that activation of CB_2 produces antinociceptive effects in persistent pain states. Importantly, CB_2-selective agonists such as HU308 and AM1241 lack centrally mediated side effects associated with activation of CB_1 receptors, including hypoactivity, hypothermia and catalepsy (Hanus *et al*., 1999; Malan *et al*., 2001). Such observations have led support to the view that CB_2 agonists would be unlikely to be psychoactive or addictive. The absence of central nervous system (CNS) side effects is consistent with the relative paucity of CB_2 receptors in brain of naive animals (Munro *et al*., 1993; Galiègue *et al*., 1995; Zimmer *et al*., 1999; Buckley *et al*., 2000). CB_2 receptors are expressed predominantly, but not exclusively outside the CNS (Van Sickle *et al*., 2005; Beltramo *et al*., 2006), where they are localized extensively to cells of the immune system. These immune cells include mast cells, B cells, T4 and T8 cells, microglial cells, macrophages, natural killer cells and to a lesser extent monocytes and polymorphonuclear neutrophils (Facci *et al*., 1995; Howlett *et al*., 2004; Maresz *et al*., 2005). CB_2 receptors have been identified in microglial cultures (Walter *et al*., 2003; Beltramo *et al*., 2006) and occur in immune tissues at levels 10–100 times greater than the CB_1 receptor (Facci *et al*., 1995; Galiègue *et al*., 1995). An emerging literature implicates a role for neuroimmune interactions in contributing to the development or maintenance of pathological pain states (for review see DeLeo and Yezierski, 2001). However, the mechanism by which CB_2 receptor activation may modulate these interactions remains poorly understood.

Cannabinoid receptor pharmacology

Activation of CB_2 receptors inhibits adenylyl cyclase (Slipetz *et al*., 1995; Di Marzo and De Petrocellis, 2006) and activates mitogen-activated protein kinase (Bouaboula *et al*., 1996; Di Marzo and De Petrocellis, 2006) through binding of the α-subunit of the $G_{i/o}$ protein. In contrast to CB_1 receptors, CB_2 receptors do not couple to calcium-Q or inward-rectifying potassium channels (Felder *et al*., 1995). Agonist binding to CB_1 receptors, by contrast, suppresses calcium and activates inward-rectifying potassium conductances—effects associated with depression of neuronal excitability and transmitter release. Thus, differences in receptor distribution and signal transduction mechanisms are likely to account for the relative absence of the CNS side effects induced by CB_2 agonists. These considerations suggest that novel pharmacotherapies targeting CB_2 receptors may have considerable therapeutic potential.

Significant drug discovery efforts have been directed towards developing and characterizing CB_2-selective agonists (see Figure 1) both *in vitro* (see Table 1) and *in vivo* (see Tables 2–5). These efforts have sought to evaluate and validate the CB_2 receptor as an analgesic target. HU308 (4-[4-(1,1-diemethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol) was the first CB_2-selective agonist exhibiting low affinity for CB_1 to be synthesized (Hanus *et al*., 1999). HU308 exhibits anti-inflammatory and peripheral antihyperalgesic properties, which are reversed by the CB_2 antagonist SR144528 but not by the CB_1 antagonist SR141716A (Hanus *et al*., 1999). HU308 fails to show CNS activity in a tetrad of behavioural tests, which assess cardinal signs of CB_1 receptor activation associated with Δ^9-tetrahydrocannabinol (Gaoni and Mechoulam, 1971), the psychoactive ingredient in cannabis.

AM1241 (2-iodo-5-nitro-phenyl)-[1-(1-methyl-piperidin-2-ylmethyl)-1*H*-indol-3-yl]-methanone) (Ibrahim *et al*., 2003) was similarly shown to lack CNS side effects in the tetrad, but nonetheless produced peripheral-mediated antinociception in otherwise naive animals (see Table 1). AM1241 induces CB_2-mediated antihyperalgesic effects in multiple models of persistent nociception, including those induced by tissue and nerve injury (see Tables 2–5). AM1241 stimulates the release of β-endorphin from skin keratinocytes (Ibrahim *et al*., 2005), suggesting that μ-opioid receptors contribute to antinociceptive effects of AM1241, but not necessarily other CB_2 agonists, that are observed in otherwise naive animals. However, whether or not β-endorphin release contributes to AM1241-mediated antihyperalgesic efficacy in models of persistent nociception has not been evaluated.

AM1241 has recently been shown to behave as a protean agonist at the CB_2 receptor *in vitro*, suggesting that functional efficacies displayed by AM1241 *in vitro* depend upon the level of receptor constitutive activities exhibited in the assay system (Yao *et al*., 2006). For example, AM1241 behaves as a neutral antagonist in FLIPR and cyclase assays and as a partial agonist in ERK (or mitogen-activated protein) kinase assays (Yao *et al*., 2006). However, at lower forskolin concentrations, AM1241 behaved as a partial agonist in the cyclase assay (Yao *et al*., 2006). Such factors may contribute to complexities (see Bingham *et al*., 2007) of *in vivo* actions of AM1241. More work is necessary to determine the signal transduction pathways implicated in the antihyperalgesic effects of AM1241. This review characterizes *in vivo* actions of AM1241 that are blocked by a CB_2 antagonist. Therefore, AM1241 will be referred to in the present work as a CB_2 agonist.

JWH-133 ((6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[b,d]pyran) is a well-characterized CB_2 agonist (Huffman *et al*., 1999; Jonsson *et al*., 2006), which inhibits both inflammatory and neuropathic hyperalgesia (see Tables 3 and 5) through a CB_2-selective mechanism. The CB_2 agonist GW405833 (2,3-dichloro-phenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-yl-ethyl)-indol-1-yl]-methanone) (Valenzano *et al*., 2005) is identical to the CB_2 agonist referred to as L768242 (1-(2,3-dichlorobenzoyl)-2-methyl-3-(2-[1-morpholino]ethyl)-5-methoxyindole) (Huffman, 2000). Here, we will refer to this

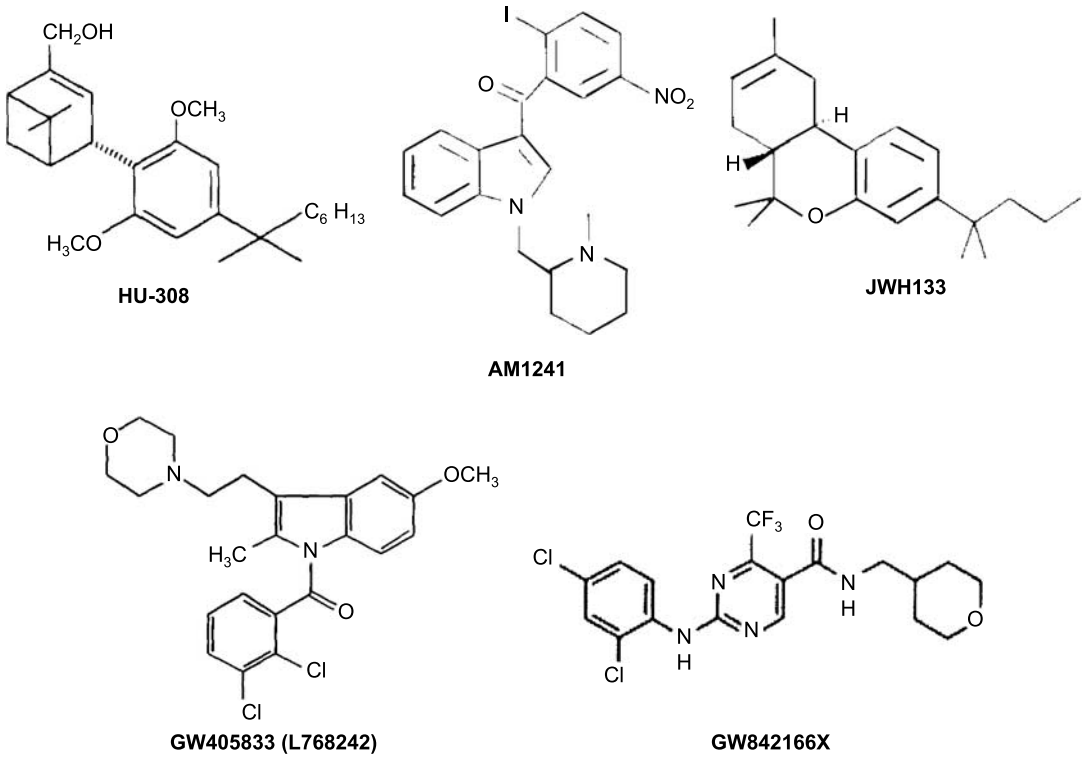

Figure 1 Chemical structures of cannabinoid CB_2-selective agonists evaluated in Tables 1–5.

compound using the nomenclature employed in the original research article, with the other common name indicated in parentheses, to emphasize that these names refer to a single compound. GW405833 (L768242) exhibits anti-inflammatory and antihyperalgesic properties (Tables 2–5). The chemical structures of the CB_2 agonists reviewed here are shown in Figure 1. The chemical structures of cannabinoid CB_1 and CB_2 antagonists are shown in Figure 2.

Nonselective cannabinoid agonists

CP55,940 and WIN55,212-2 are potent cannabinoid agonists that bind with high affinity to both CB_1 and CB_2 (Lan *et al.*, 1999; Huffman, 2000; Palmer *et al.*, 2002). These agonists suppress pain behaviour in different animal models of acute, tissue and nerve injury-induced nociception (for review see Walker and Hohmann, 2005). However, it is important to emphasize that the pharmacological profile exhibited by cannabinoid agonists *in vivo* may differ from the pharmacological profile demonstrated *in vitro* (for example, that suggested by their *in vitro* binding affinities). Despite possessing high affinity for CB_2 *in vitro*, mixed CB_1/CB_2 agonists do not necessarily exhibit pharmacological properties in different pain models that are typical of other CB_2-selective agonists *in vivo*. For example, antinociception induced by CP55,940, administered systemically, can be largely attributed to CB_1 (Choong *et al.*, 2007; Pryce and Baker, 2007). However, a role for CB_2 in contributing to CP55,940-mediated antinociception has recently been described in both acute (tail flick assay) and neuropathic (spinal nerve ligation) pain models (Scott *et al.*, 2004), whereas the antihyperalgesic effects of CP55,940 have solely been attributed to CB_1 in an inflammatory pain model (Choong *et al.*, 2007). Suppression of neuropathic nociception induced by systemically administered WIN55,212-2 has been shown to be mediated by CB_1 (Herzberg *et al.*, 1997; Bridges *et al.*, 2001) and not by CB_2 (Bridges *et al.*, 2001). Studies employing intraplantar injections of WIN55,212-2 also confirm a role for CB_1 receptors in suppressing neuropathic nociception following local administration; however, a role for CB_2 mechanisms in contributing to the antihyperalgesic effects of WIN55,212-2 was not assessed (Fox *et al.*, 2001). Thus, it is noteworthy that both CB_1 and CB_2 receptors have been implicated in the antihyperalgesic effects of locally (intraplantar) administered WIN55,212-2 in the carrageenan model of inflammatory nociception (Nackley *et al.*, 2003b). Indeed, agonists that act on both CB_1 and CB_2 receptors *in vitro* can produce *in vivo* pharmacological effects, wherein activity at CB_1 predominates (Dyson *et al.*, 2005); these effects may differ with the route of agonist administration employed (systemic versus local) or nociceptive state (acute, tissue injury or nerve injury). Therefore, the present review will be restricted to evaluation of *in vivo* pharmacological effects of CB_2-selective agonists that exhibit minimal affinity at CB_1 (see Table 1). Here, we review preclinical studies that assess the role of CB_2 receptor activation in suppressing pain in animal models of acute, inflammatory and neuropathic nociception using the best characterized CB_2-selective agonists available to date. The antinociceptive effects of mixed cannabinoid agonists are reviewed elsewhere (Walker and Hohmann, 2005).

Table 1 *In vitro* binding profile of cannabinoid CB_2 agonists and CB_1 and CB_1 antagonists

Compound		*CB_1*			*CB_2*	*Probe*	*Reference*
HU-308	CB_2 agonist	$K_i > 10\,\mu M$	Rat brain	$K_i = 22.7 \pm 3.9$ nM	Transfected cells	[^{3}H]HU-243	Devane *et al.*, 1992; Mechoulam *et al.*, 1995
AM1241	CB_2 agonist	$K_i = 280 \pm 41$ nM	Rat brain	$K_i = 3.4 \pm 0.5$ nM	Mouse spleen	[^{3}H]CP55,940	Ibrahim *et al.*, 2003
JWH-133	CB_2 agonist	$K_i = 677 \pm 132$ nM	Rat brain	$K_i = 3.4 \pm 1.0$ nM	Human embryonic kidney 293 cells	[^{3}H]CP55,940	Huffman *et al.*, 1999
GW405833	CB_2 agonist	$K_i = 2043 \pm 183$ nM	Cos-7 cells	$K_i = 14 \pm 6$ nM	Cos-M6 cells	[^{3}H]WIN55212-2	Slipetz *et al.*, 1995; Gallant *et al.*, 1996
(L768242)		$K_i = 273 \pm 42.6$ nM	Rat brain	$K_i = 3.6 \pm 1.1$ nM	Rat spleen	[^{3}H]CP55,940	Valenzano *et al.*, 2005
GW842166 X	CB_2 agonist	Not available		Not available		Not available	Giblin *et al.*, 2007
SR141716A	CB_1 antagonist	$K_i = 2$ nM	Rat brain	$K_i > 1000$ nM	Mouse vas deferens	[^{3}H]CP55,940	Rinaldi-Carmona *et al.*, 1995
AM251	CB_1 antagonist	$K_i = 7.5$ nM	Rat forebrain	$K_i = 2290$ nM	Mouse spleen	[^{3}H]CP55,940	Gatley *et al.*, 1997; Lan *et al.*, 1999; Palmer *et al.*, 2002
SR144528	CB_2 antagonist	$K_i = 305 \pm 44$ nM	Rat brain	$K_i = 0.30 \pm 0.38$ nM	Rat spleen	[^{3}H]CP55,940	Rinaldi-Carmona *et al.*, 1998
AM630	CB_2 antagonist	$K_i = 5152 \pm 567$ nM	CHO cells	$K_i = 31.2 \pm 12.4$ nM	CHO cells	[^{3}H]CP55,940	Ross *et al.*, 1999

Table 2 Antinociceptive effects of cannabinoid CB_2 agonists in animal models of acute pain

Pain model	*Drugs*	*Route of administration*		*Pharmacological specificity*		*Antinociception*	*Mediated by*		*Studies*
		Systemic	*Local*	*CB_1 (local/systemic)*	*CB_2 (local/systemic)*		*CB_1*	*CB_2*	*Reference*
Acute									
● Plantar	HU-308	40 mg kg^{-1}, i.p.	—	NA	NA	No	NA	NA	Hanus *et al.*, 1999
● Plantar	AM1241	0.033–0.33 mg kg^{-1}, i.p.	0.33–3.3 mg kg^{-1}, i.paw	Not blocked by AM251; 1 mg kg^{-1}, i.p.; 330 mg kg^{-1}, i.paw	Blocked by AM630; 1 mg kg^{-1} i.p.; 100 μg kg^{-1} i.paw	Yes	No	Yes	Malan *et al.*, 2001
● Plantar	AM1241	100 μg kg^{-1}, i.p.	—	See Malan *et al.*, 2001		Yes	No	Yes	Ibrahim *et al.*, 2005
● Plantar/tail flick	GW405833 (L768242)	3–30 mg kg^{-1}, i.p.	—	NA	NA	No	NA	NA	Valenzano *et al.*, 2005
● Plantar/tail flick	GW405833 (L768242)	100 mg kg^{-1}, i.p.	—	NT	NT	Yes	NT	NT	Valenzano *et al.*, 2005
● Plantar/tail flick	GW405833 (L768242)	100 mg kg^{-1}, i.p.	—	Antinociceptive effect in both $CB_2^{+/+}$ and $CB_2^{-/-}$ mice		Yes	NT	No	Whiteside *et al.*, 2005
● Plantar/tail flick	AM1241	0.3–10 mg kg^{-1}, i.p.	—	Antinociceptive effect in $CB_2^{+/+}$ but not in $CB_2^{-/-}$ mice		Yes Plantar > TF	NT	Yes	Ibrahim *et al.*, 2006
● Hot plate/tail flick	AM1241	1–10 mg kg^{-1}, i.p.	—	NA	NA	No	NA	NA	Bingham *et al.*, 2007

Abbreviaions: i.p., intraperitoneal; i.paw, dorsal surface of the paw; NA, not applicable; NT, not tested; TF, tail flick.
● Tested on rats □ and mice ■.

Table 3 Antinociceptive effects of cannabinoid CB_2 agonists in the carrageenan model of inflammation

Pain model	*Drugs*	*Route of administration*		*Pharmacological specificity*		*Antinociception*	*Mediated by*		*Studies*
		Systemic	*Local*	*CB_1 (local/systemic)*	*CB_2 (local/systemic)*		*CB_1*	*CB_2*	*Reference*
Inflammatory									
●Carrageenan i.pl. post	GW405833 (L768242)	0.3–10 mg kg^{-1}, i.p.	—	NT	Blocked by SR144528; 3 mg kg^{-1}, i.p.	Yes WB	NT	Yes	Clayton *et al.*, 2002
●Carrageenan i.pl. post	AM1241	33–330 μg kg^{-1}, i.p.	33 μg kg^{-1}, i.pl.	Not blocked by SR141716A; 1 mg kg^{-1}, i.p.	Blocked by SR144528; 1 mg kg^{-1}, i.p.	Yes M and T Fos	No	Yes	Nackley *et al.*, 2003a
●Carrageenan i.paw pre	AM1241	0.1–1 mg kg^{-1}, i.p.	1–4 mg kg^{-1}, i.paw	Not blocked by AM251; 300 μg kg^{-1}, i.p.; 300 mg kg^{-1}, i.paw	Blocked by AM630; 100 μg kg^{-1} i.p.; 100 μg kg^{-1}, i.paw	Yes T	No	Yes	Quartilho *et al.*, 2003
●Carrageenan i.pl. post	AM1241	330 μg kg^{-1}, i.v.	33 or 330 μg kg^{-1}, i.pl.	Not blocked by SR141716A; 1 mg kg^{-1}, i.v.	Blocked by SR144528; 1 mg kg^{-1}, i.v.	Yes NP (E) Inf > Noninf	No	Yes	Nackley *et al.*, 2004
●Carrageenan i.pl. pre	JWH-133	—	5–15 μg in 50 μl, i.pl.	Not blocked by SR141716A; 10 μg in 50 μl i.pl.	Blocked by SR144528; 10 μg in 50 μl i.pl.	Yes NP (M) Inf ≈ Noninf	No	Yes	Elmes *et al.*, 2004
●Carrageenan i.pl. pre	JWH-133	0.3–10 mg kg^{-1}, s.c.	—	Not blocked by SR141716A; 3 mg kg^{-1}, s.c.	Blocked by SR144528; 3 mg kg^{-1}, s.c.	Yes WB	No	Yes	Elmes *et al.*, 2005
●Carrageenan i.pl. pre	AM1241	—	33 μg kg^{-1}, i.pl.	Not blocked by SR141716A; 33 μg kg^{-1}, i.pl.	Blocked by SR144528; 33 μg kg^{-1}, i.pl.	Yes M and T M > T	No	Yes	Gutierrez *et al.*, 2007
●Carrageenan i.pl. pre	AM1241	1–10 mg kg^{-1}, i.p.	—	NT	Blocked by AM630; 1 mg kg^{-1}, i.p.	Yes T	NT	Yes	Bingham *et al.*, 2007

Abbreviations: Fos, suppression of carrageenan-evoked spinal Fos protein in lamina I, II and V, VI; Inf, inflamed; i.p., intraperitoneal; i.paw, dorsal surface of the paw; i.pl., intraplantar; i.v., intravenous; E, transcutaneous electical stimulation; M, mechanical; Noninf, noninflamed; NP, neurophysiological evidence from extracellular recordings of spinal wide dynamic range neurons; NT, not tested; post, carrageenan injected after drugs; pre, carrageenan injected before drugs; s.c., subcutaneous; T, thermal; WB, weight bearing.
●Tested on rats □ .

Table 4 Antinociceptive effects of cannabinoid CB_2 agonists in animal models of inflammatory pain

Pain model	*Drugs*	*Route of administration*		*Pharmacological specificity*		*Antinociception*	*Mediated by*		*Studies*
		Systemic	*Local*	*CB_1 (local/systemic)*	*CB_2 (local/systemic)*		*CB_1*	*CB_2*	*Reference*
Inflammatory									
● Capsaicin i.paw post	AM1241	0.03–0.3 mg kg^{-1}, i.p.	—	Not blocked by AM251; 300 μg kg^{-1}, i.p.	Blocked by AM630; 100 μg kg^{-1}, i.p.	Yes T	No	Yes	Quartilho *et al.*, 2003
● Capsaicin i.pl. post	AM1241	33 or 330 μg kg^{-1}, i.p.	33 μg kg^{-1}, i.pl.	Not blocked by SR141716A; 1 mg kg^{-1}, i.p.	Blocked by SR144528; 1 mg kg^{-1}, i.p.	Yes M and T NB	No	Yes	Hohmann *et al.*, 2004
● CFA i.pl. pre	GW405833 (L768242)	0.01–30 mg kg^{-1}, i.p.	—	NT	NT	Yes M	NT	NT	Valenzano *et al.*, 2005
● CFA i.pl. pre	GW405833 (L768242)	3–30 mg kg^{-1}, i.p.	—	Antinociceptive effect in $CB_2^{+/+}$ but not in $CB_2^{-/-}$ mice		Yes M	NT	Yes	Valenzano *et al.*, 2005; Whiteside *et al.*, 2005
● CFA i.pl. pre	GW842166X	0.3–1 mg kg^{-1}, o.	—	NT	Blocked by AM630; 15 mg kg^{-1}, i.p.	Yes WB	NT	Yes	Giblin *et al.*, 2007
● Formalin i.pl post	HU-308	50 mg kg^{-1}, i.p.	—	NT	Blocked by SR144528; 0.5 mg kg^{-1}, i.p.	Yes LP	NT	Yes	Hanus *et al.*, 1999
● Formalin i.pl. post	AM1241	0.3–3 mg kg^{-1}, i.v.	—	NT	Blocked by SR144528; 1 mg kg^{-1}, i.p.	Yes LP	NT	Yes	Beltramo *et al.*, 2006
● Formalin i.pl. post	L768242 (GW405833)	3–10 mg kg^{-1}, i.v.	—	NT	Blocked by SR144528; 1 mg kg^{-1}, i.p.	Yes LP	NT	Yes	Beltramo *et al.*, 2006
● Acid arachidonic [a]ear post	HU-308	50 mg kg^{-1}, i.p.	—	Not blocked by SR141716A; 5 mg kg^{-1}, i.p.	Blocked by SR144528; 1 mg kg^{-1}, i.p.	Yes	No	Yes	Hanus *et al.*, 1999

Abbreviations: CFA, complete Freund's adjuvant; i.paw, dorsal surface of the paw; i.p., intraperitoneal; i.pl., intraplantar; i.v., intravenous; LP, late phase; M, mechanical; NB, nocifensive behaviour; NT, not tested; o., oral; post, capsaicin/CFA/formalin injected after drugs; pre, capsaicin/CFA/formalin injected before drugs; T, thermal; WB, weight bearing.
[a]Applied to the inner surface of one ear.
●Tested on rats □ and mice ■.

Table 5 Antinociceptive effects of cannabinoid CB_2 agonists in animal models of neuropathic pain

Pain model	*Drugs*	*Route of administration*		*Pharmacological specificity*		*Antinociception*	*Mediated by*		*Studies*
		Systemic	*Local*	*CB_1 (local/systemic)*	*CB_2 (local/systemic)*		*CB_1*	*CB_2*	*Reference*
Neuropathic									
● SNL	AM1241	100–3000 μg kg^{-1}, i.p.	—	Not blocked by AM251; 300 μg kg^{-1}, i.p.	Blocked by AM630; 300 μg kg^{-1}, i.p.	Yes M and T	No	Yes	Ibrahim *et al.*, 2003
■ SNL	AM1241	1–3 mg kg^{-1}, i.p.	—	Not blocked by AM251; 300 μg kg^{-1} i.p. in $CB_1^{+/+}$ and $CB_1^{-/-}$ mice	Blocked by AM630; 1 mg kg^{-1} i.p. in $CB_1^{+/+}$ and $CB_1^{-/-}$ mice	Yes M and T	No	Yes	Ibrahim *et al.*, 2003
● SNL	JWH-133	—	5–15 μg in 50 μl i.pl.	NT	Blocked by SR144528; 10 μg in 50 μl i.pl.	Yes NP	NT	Yes	Elmes *et al.*, 2004
● SNL	JWH-133	8–486 ng in 50 μl spinal	—	Not blocked by SR141716A; 0.01 μg in 50 μl spinal	Blocked by SR144528; 0.001 μg in 50 μl spinal	Yes NP	No	Yes	Sagar *et al.*, 2005
● SNL	AM1241	3–6 mg kg^{-1}, i.v.	—	NT	Blocked by SR144528; 1–3 mg kg^{-1}, i.p.	Yes M	NT	Yes	Beltramo *et al.*, 2006
● SNL	L768242 (GW40583	10–30 mg kg^{-1}, i.p.	—	NT	NT	Yes M	NT	NT	Beltramo *et al.*, 2006
● PSNL	GW405833 (L768242)	0.01–30 mg kg^{-1}, i.p.	—	NT	NT	Yes M	NT	NT	Valenzano *et al.*, 2005
■ PSNL	GW405833 (L768242)	3–30 mg kg^{-1}, i.p.	—	NT	NT	Yes M	NT	NT	Whiteside *et al.*, 2005
● CN-V	AM1241	2.5 mg kg^{-1}, i.p.	—	Not blocked by SR141716A; 2.5 mg kg^{-1}, i.p.	Blocked by SR144528 2.5 mg kg^{-1}, i.p.	Yes M	No	Yes	Rahn *et al.*, 2007

Abbreviations: CN-V, chemotherapy-evoked neuropathy by vincristine; i.p., intraperitoneal; i.pl., intraplantar; i.v., intravenous; M, mechanical; NP, neurophysiological evidence from extracellular recordings of spinal wide dynamic range neurons; NT, not tested; PSNL, partial sciatic nerve ligation; SNL, spinal nerve ligation; T, thermal.
●Tested on rats □ and mice ■.

Acute pain

Cannabinoids induce antinociceptive effects through spinal, supraspinal and peripheral mechanisms (Martin *et al.*, 1995; Pertwee, 2001; Hohmann, 2002; Walker and Hohmann, 2005). Recent studies suggest that some, but not all, CB_2-selective agonists induce antinociception in tests of acute pain in otherwise naive animals. The magnitude of the observed antinociception may differ with the assay for acute nociception and agonist and dose employed (see Table 2). Systemic (intraperitoneal) and local (intraplantar) administration of AM1241 produces a thermal antinociceptive effect in the plantar test in otherwise naive animals (Malan *et al.*, 2001; but see Bingham *et al.*, 2007). This test measures the latency for animals to remove their paws from a radiant heat source that is focused onto the plantar surface of the paw through the floor of a glass platform. This antinociceptive effect was mediated by CB_2 receptors because it was antagonized by the CB_2-selective antagonist AM630, administered systemically or locally into the dorsal surface of the paw. By contrast, systemic or local administration of the CB_1 antagonist AM251 did not alter AM1241-induced antinociception. AM1241 induces antinociception in the plantar test in rats (Malan *et al.*, 2001) and mice (Ibrahim *et al.*, 2006). The ability of AM1241 to inhibit acute nociception in the hot plate and tail flick tests is also lost in $CB_2^{-/-}$ mice, confirming a role for CB_2 receptors in these actions (Ibrahim *et al.*, 2006) (see Table 2). These studies also reveal that AM1241 is less efficacious in producing antinociception in the spinally mediated tail flick test relative to the plantar test, which assesses latency to paw withdrawal. By contrast, systemic administration of HU308 and GW405833 (L768242) failed to induce antinociception in the hot plate (Hanus *et al.*, 1999; Valenzano *et al.*, 2005) and tail flick (Valenzano *et al.*, 2005) tests.

Systemic administration of a high dose ($100\,mg\,kg^{-1}$) of GW405833 (L768242) elevated thermal paw withdrawal latencies in the hot plate and tail flick test in rats (Valenzano *et al.*, 2005). However, these effects are unlikely to be attributed to activation of CB_2 receptors; the same dose ($100\,mg\,kg^{-1}$) of GW405833 (L768242) induced antinociceptive effects in both $CB_2^{-/-}$ and $CB_2^{+/+}$ mice and induced motor ataxia (Valenzano *et al.*, 2005; Whiteside *et al.*, 2005). Interestingly, antihyperalgesic doses of all three compounds—AM1241, HU308 and GW405833 (L768242)—failed to alter locomotor activity following systemic administration. These data suggest that CB_2-selective agonists do not induce other centrally mediated effects associated with activation of CB_1. The lack of CNS side effects observed with antihyperalgesic doses of CB_2 agonists (that is, lower doses that can be specifically attributed to CB_2-specific mechanisms) may also reflect limited CNS penetration of some but certainly not all CB_2 agonists. For example, GW405833 (L768242) has been shown to penetrate the CNS (Valenzano *et al.*, 2005). Complete pharmacokinetic profiles for new and existing CB_2 agonists are needed to better address this issue.

More work is also necessary to verify that the antinociceptive effects of AM1241 (i.p.) in modulating acute nociception represent a class effect typical of other CB_2 agonists. Electrophysiological studies employing transcutaneous electrical stimulation reveal that AM1241 preferentially suppresses the mechanism by which spinal neurons are

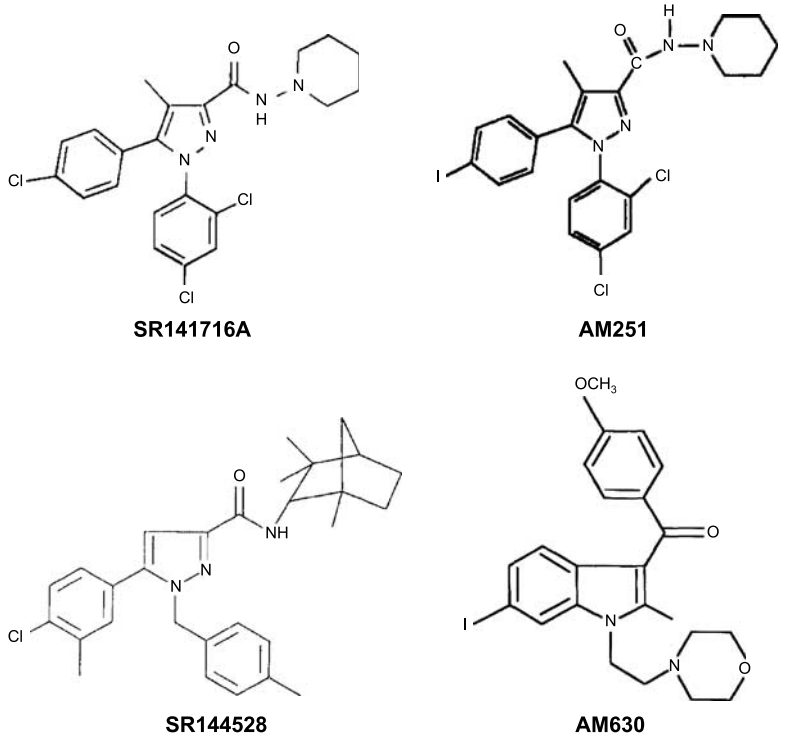

Figure 2 Chemical structures of cannabinoid CB_1 (SR141716A, AM251) and CB_2 (SR144528, AM630) antagonists.

sensitized; this suppression is more pronounced in the presence compared to the absence of inflammation (Nackley *et al*., 2004). Thus, it is noteworthy that three structurally distinct CB_2 agonists (AM1241, GW405833 (L768242) and HU308) suppress acute responses to mechanical stimulation following tissue injury induced by hindpaw incision (LaBuda *et al*., 2005). Hindpaw incision induces microglial and astrocytic activation (Romero-Sandoval and Eisenach, 2007) as well as tactile allodynia (LaBuda *et al*., 2005). Hindpaw incision-induced tactile allodynia was suppressed by all three CB_2 agonists. The antiallodynic effects of HU308 were also blocked by SR144528, consistent with mediation by CB_2. Consequently, a better understanding of the mechanisms involved in CB_2-mediated antinociceptive effects as well as the signal transduction mechanisms underlying these actions is required to understand how activation of CB_2 modulates nociceptive responding in the presence versus absence of pathological pain states.

Persistent inflammatory nociception

Cannabinoids are antinociceptive in tissue injury models of persistent pain. Behavioural, electrophysiological and neurochemical studies all support a role for CB_2 receptor activation in modulating inflammatory nociception. Effects of CB_2-selective agonists in different inflammatory pain models (carrageenan, capsaicin, complete Freund's adjuvant, formalin and arachidonic acid) will be discussed separately (see Tables 3 and 4) because mechanisms underlying the development of hyperalgesia, allodynia and spontaneous pain in distinct models of tissue injury-induced nociception differ.

Carrageenan model

Intraplantar injection of carrageenan produces paw swelling (oedema) and hyperalgesia (Hargreaves *et al*., 1988) and induces expression of Fos, a nonspecific marker of neuronal activation (Honore *et al*., 1995). Systemic or local (intraplantar) administration of AM1241 suppresses the development of behavioural sensitization to both mechanical and thermal stimulation in the carrageenan model of inflammation (Nackley *et al*., 2003a). These antihyperaglesic effects were mediated by CB_2 receptors because they were blocked by the CB_2 antagonist SR144528, but not by the CB_1 antagonist SR141716A (Nackley *et al*., 2003a). AM1241 also suppresses spinal Fos expression, a marker of neuronal activation, in the carrageenan model of inflammation; this suppression was similarly blocked by coadministration of AM1241 with SR144528 (Nackley *et al*., 2003a). AM1241 suppressed carrageenan-evoked Fos protein expression in a lamina-specific manner. CB_2-mediated suppressions of carrageenan-evoked Fos protein expression were observed in the superficial (lamina I, II) and neck region (lamina V, VI) of the dorsal horn, spinal cord regions associated primarily with the termination of nociceptive primary afferents. By contrast, AM1241 did not alter Fos protein expression in the nucleus proprius (lamina III, IV) or ventral horn (Nackley *et al*., 2003a). These data are consistent with the hypothesis that antihyperalgesic effects of AM1241 in models of inflammatory nociception reflect a suppression of inflammation-evoked neuronal activation.

Local administration of AM1241 also attenuates the maintenance of thermal (Quartilho *et al*., 2003; Gutierrez *et al*., 2007) and mechanical (Gutierrez *et al*., 2007) hypersensitivity induced by hindpaw injection of carrageenan. These effects are blocked by CB_2-selective antagonists such as AM630 or SR144528. Moreover, local injections of SR144528 but not SR141716A block the antihyperalgesic effects of locally administered AM1241 in a model of established (18 h post injection) carrageenan inflammation; these antihyperalgesic effects are observed with multiple modalities of stimulation (mechanical, thermal) (Gutierrez *et al*., 2007). The ability of intraplantar administration of SR144528 to block the antihyperalgesic effects of locally administered AM1241 cannot be attributed to nonspecific actions of the drug at CB_1 receptors; under identical conditions, local administration of SR141716A, but not SR144528, blocked the antihyperalgesic effects of locally administered ACEA, a CB_1-selective agonist (Gutierrez *et al*., 2007). This latter study also revealed more robust effects of AM1241 in suppressing responses to mechanical as opposed to thermal stimulation after the establishment of carrageenan inflammation.

Intravenous or local hindpaw administration of AM1241 also suppresses neuronal sensitization recorded in spinal nociceptive neurons during the development of carrageenan inflammation (Nackley *et al*., 2004). This observation suggests a neurophysiological mechanism capable of mediating the antihyperalgesic effects of AM1241. Spinal neuronal excitability was induced by applying trains of electrical stimulation to the peripheral receptive field in the ipsilateral hindpaw in the absence or presence of carrageenan inflammation. During the development of carrageenan inflammation, preemptive administration of AM1241 preferentially suppressed C fibre-mediated afterdischarge responses and windup—electrophysiological effects attributed to C fibre-mediated sensitization of wide dynamic range neurons (Nackley *et al*., 2004). The AM1241-induced suppression of electrically evoked responses was blocked by the CB_2 antagonist SR144528, but not by the CB_1 antagonist SR141716A (Nackley *et al*., 2004). Moreover, activity evoked in purely non-nociceptive neurons (that is, A-β fibre-mediated responses recorded in low threshold mechanosensitive cells) was unaffected. Thus, behavioural, electrophysiological and neurochemical studies suggest that AM1241 preferentially suppresses neuronal sensitization that is observed in the presence compared to the absence of an inflammatory pain state. These observations are also consistent with the ability of intraplantar injections of JWH-133 to suppress mechanically evoked responses of wide dynamic range neurons in carrageenan-treated rats through a CB_2-specific mechanism; this electrophysiological response was blocked by local administration of the CB_2 antagonist SR144528 but not by the CB_1 antagonist SR141716A (Elmes *et al*., 2004).

Carrageenan inflammation also decreases weight bearing in the inflamed paw. Thus, it is noteworthy that both GW405833 (L768242) and JWH-133, administered systemically, reverse this effect. GW405833 (L768242) and JWH-133, cannabinoid CB_2 agonists from different chemical classes, increase weight bearing in the carrageenan-inflamed

paw through a mechanism that is dependent upon CB_2 receptor activation (Clayton *et al*., 2002; Elmes *et al*., 2005). Like AM1241 (Quartilho *et al*., 2003; Nackley *et al*., 2004), both GW405833 (L768242) and JWH-133 also decrease carrageenan-evoked peripheral oedema (Clayton *et al*., 2002; Elmes *et al*., 2005). Thus, the available data suggest that multiple CB_2-selective agonists suppress inflammatory nociception and peripheral oedema induced by hindpaw carrageenan administration; these effects are observed in behavioural, electrophysiological and neurochemical studies, involve multiple stimulus modalities (mechanical, thermal), are observed following systemic or local agonist administration and are blocked by CB_2 but not CB_1 antagonists (see Table 3). The ability of CB_2 agonists to suppress persistent nociception in other tissue-injury models of persistent pain is summarized in Table 4.

Capsaicin model

Intradermal administration of capsaicin, the pungent ingredient in hot chilli peppers, induces hypersensitivity to mechanical and thermal stimulation as well as spontaneous pain (Gilchrist *et al*., 1996). Hyperalgesia evoked by capsaicin treatment refers to an increase in pain behaviour evoked by suprathreshold stimuli and/or lowered threshold for pain (Gilchrist *et al*., 1996). Primary hyperalgesia, especially that elicited by noxious thermal stimulation, is mediated in part by sensitization of C-fibre mechanoheat (polymodal) nociceptors (LaMotte *et al*., 1992; Torebjörk *et al*., 1992). Secondary (mechanical) hyperalgesia is observed in surrounding uninjured tissue and involves sensitization of the CNS (Baumann *et al*., 1991; LaMotte *et al*., 1992) as well as nociceptor sensitization (Serra *et al*., 2004).

AM1241, administered systemically, induced a dose-dependent suppression of capsaicin-evoked thermal hyperalgesia and spontaneous pain behaviour (Quartilho *et al*., 2003; Hohmann *et al*., 2004). These antihyperalgesic effects were mediated by CB_2 receptors because they were antagonized by AM630 (Quartilho *et al*., 2003) and SR144528 (Hohmann *et al*., 2004). Both local (intraplantar) and systemic (intraperitoneal) administration of AM1241 suppresses mechanical hyperalgesia and allodynia as well as thermal hypersensitivity evoked by intradermal capsaicin injection (Hohmann *et al*., 2004). The suppressive effects of AM1241 were dose-dependent and antagonized by SR144528, but not SR141716A. Moreover, capsaicin-evoked nocifensive behaviour (licking, lifting and failure to bear weight on the injected paw) was also blocked by AM1241 through a CB_2-specific mechanism (Hohmann *et al*., 2004). The antihyperalgesic effects of AM1241 were mediated, at least in part, by a local site of action; AM1241 injected into the capsaicin-injected paw suppressed capsaicin-evoked hypersensitivity to mechanical and thermal stimulation, whereas injection of the same dose into the contralateral (capsaicin-untreated) paw was inactive (Hohmann *et al*., 2004).

Complete Freund's adjuvant model

Intraplantar administration of complete Freund's adjuvant in rodents induces peripheral oedema as well as hypersensitivity to mechanical and thermal stimulation (Ren and Dubner, 1999). Inflammation appears approximately 2 h following injection of complete Freund's adjuvant, produces its maximal effect after 6–8 h and can persist for weeks following injection (Ren and Dubner, 1999; Walker *et al*., 2003). GW405833 (L768242), administered systemically, suppressed the development of adjuvant-induced tactile allodynia and mechanical hyperalgesia in a dose-dependent manner. This suppression was observed in both rats and mice (Valenzano *et al*., 2005; Whiteside *et al*., 2005). Although pharmacological specificity of GW405833 (L768242) was not assessed in rats, CB_2 receptors are nonetheless likely to mediate the observed suppression of mechanical hypersensitivity (Valenzano *et al*., 2005; Whiteside *et al*., 2005). GW405833 (L768242) suppressed adjuvant-induced mechanical hypersensitivity in $CB_2^{+/+}$ mice, but these antihyperalgesic effects were absent in $CB_2^{-/-}$ mice. Moreover, another CB_2 agonist, GW842166X (2-[(2,4-dichlorophenyl)amino]-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide), administered orally, fully reversed complete Freund's adjuvant-induced hyperalgesia when weight bearing was used to assess behavioural sensitization. This effect was blocked by AM630, albeit at a high dose (15 mg kg^{-1}, i.p.), and possible mediation by CB_1 was not assessed (Giblin *et al*., 2007).

A better understanding of the mechanism of action of CB_2-selective agonists has recently been obtained using GW405833 (L768242) and the complete Freund's adjuvant model of inflammatory pain (see Table 4). Whiteside *et al*. (2005) evaluated the ability of the opioid antagonist naltrexone to block the antihyperalgesic effects of GW405833 (L768242) in mice subjected to adjuvant-induced inflammation of the hindpaw. Naltrexone was ineffective in blocking the antihyperalgesic effects of GW405833 (L768242) (Whiteside *et al*., 2005). From this later study, it can be concluded that CB_2-mediated antihyperalgesic effects of GW405833 (L768242) are not dependent upon the release of endogenous opioids (Whiteside *et al*., 2005). By contrast, AM1241 releases β-endorphin from skin keratinocytes following activation of CB_2 receptors in otherwise naive animals (Ibrahim *et al*., 2005). It is noteworthy, therefore, that the antinociceptive efficacy of AM1241 (i.p.), the only CB_2 agonist shown to date, to produce antinociception in an acute pain model (the plantar test) in otherwise naive animals is also lost in μ-opioid receptor knockout mice (Ibrahim *et al*., 2005). Thus, the available data suggest that multiple CB_2-selective agonists suppress behavioural sensitization induced by complete Freund's adjuvant administration in both rats and mice through a CB_2-specific mechanism. These effects are blocked by CB_2 antagonists and are absent in $CB_2^{-/-}$ mice. Moreover, antihyperalgesic efficacy of CB_2-selective agonists in this model does not require opioid receptor activation or mobilization of β-endorphin. Importantly, the available data collectively suggest that β-endorphin release and μ-opioid receptor sensitivity are not a class effect associated with all CB_2-selective agonists.

Formalin model

The formalin test is a well-established model of persistent pain characterized by a transient, biphasic pattern of pain

behaviour. The early phase is characterized by acute activation of C and Aδ fibres. The late phase involves an inflammatory reaction in peripheral tissue (Tjölsen *et al*., 1992), the development of CNS sensitization (Coderre and Melzack, 1992; Coderre and Katz, 1997) and additionally involves activation of primary afferent nociceptors (Puig and Sorkin, 1996). CB_2 agonists are antinociceptive in the formalin test (see Table 4). The antinociceptive effect of HU308 was restricted to the late phase of the formalin test (Hanus *et al*., 1999), which is associated with CNS sensitization. Both AM1241 and the CB_2-selective agonist L768242 (GW405833), administered intravenously, similarly reduced the late, but not the early phase, of formalin pain. The antinociceptive effect of each agonist was also dependent upon CB_2 receptor activation (Beltramo *et al*., 2006). These observations are consistent with previous work demonstrating that intraplantar administration of PEA suppresses formalin-evoked pain behaviour through a mechanism that is blocked by the CB_2 antagonist SR144528 (Calignano *et al*., 1998). Intraplantar administration of PEA also preferentially suppresses spinal neuronal sensitization evoked by hindpaw formalin administration; this suppression is observed under conditions in which acute responses to non-noxious mechanical stimulation are unaffected (LoVerme *et al*., 2006). Effects of CB_2-selective agonists have not been characterized in the formalin model using electrophysiological methods, although they might be predicted to behave similarly to PEA.

Efficacy of multimodal therapies directed at CB_2 receptors and other analgesic targets (for example, enzymes catalyzing endocannabinoid deactivation) is also supported in the literature. Endogenous anandamide and PEA can be detected in paw skin, where they may engage peripheral CB_1 and CB_2 receptor subtypes (Calignano *et al*., 1998). Thus, it is noteworthy that local coadministration of PEA with exogenous anandamide (an endocannabinoid acting at CB_1/CB_2 receptors) produces a synergistic analgesic effect in both phases of the formalin test through a mechanism that involves both CB_1 and CB_2 receptor subtypes (Calignano *et al*., 1998). The combination of anandamide with ibuprofen (a nonspecific cyclooxygenase inhibitor) produced a synergistic local antinociceptive effect in both phases of the formalin test that is similarly mediated by both CB_1 and CB_2 receptors (Guindon *et al*., 2006). Endocannabinoid levels are also enhanced by the combination of anandamide with ibuprofen/rofecoxib (Guindon *et al*., 2007b). Similarly, exogenous 2-arachidonoylglycerol, an endocannabinoid acting at CB_1/CB_2 receptors, in combination with the monoacylglycerol lipase inhibitor, URB602 (an inhibitor 2-arachidonoylglycerol deactivation), produces additive antinociceptive effects (Guindon *et al*., 2007a). The effects of 2-arachidonoylglycerol were mediated by CB_2 receptors, whereas the effects of URB602 involved both CB_1 and CB_2 receptor subtypes. These studies raise the possibility that CB_2 receptors may also be targeted indirectly by inhibiting endocannabinoid deactivation, thereby elevating levels of endocannabinoids at peripheral sites where they are produced on demand in a stimulation contingent fashion. More work is necessary to determine whether such adjunctive strategies may be exploited clinically to preferentially enhance the efficacy of local antihyperalgesic mechanisms. Such adjunctive therapies may exhibit a more beneficial and circumscribed spectrum of physiological effects compared to direct agonist administration.

Arachidonic acid-induced ear oedema model

Topical administration of arachidonic acid in the ear of the mouse induces a characteristic inflammatory response (Hanus *et al*., 1999). HU308, administered intraperitoneally prior to arachidonic acid application, significantly reduced ear tissue swelling (Hanus *et al*., 1999). This anti-inflammatory effect was reduced by SR144528, consistent with mediation by CB_2 receptors (Hanus *et al*., 1999).

Nerve injury-induced nociception

Animal models of neuropathic pain have been developed to mimic symptoms associated with nerve injury observed clinically. Neuropathic pain may be induced by traumatic injury, metabolic challenges and chemotherapeutic agents (Seltzer *et al*., 1990; Polomano and Bennett, 2001; Cantón *et al*., 2004). Pharmacotherapies (for example, opioids, antidepressants and anticonvulsants) used to treat neuropathic pain produce inadequate pain relief and/or unwanted side effects. Thus, the identification of novel therapeutic approaches with limited side effect profiles remains an urgent medical need.

In behavioural studies, nonselective cannabinoid agonists reduce mechanical allodynia and thermal hyperalgesia (Herzberg *et al*., 1997; Bridges *et al*., 2001; Fox *et al*., 2001; Guindon and Beaulieu, 2006). However, the role of CB_2 receptor activation in modulation of neuropathic pain remains poorly understood. Only a small number of studies have evaluated the efficacy of CB_2-selective agonists for suppressing neuropathic nociception; these studies have employed models of neuropathic pain evoked by traumatic nerve injury (that is, partial sciatic nerve ligation and spinal nerve ligation models) and chemotherapeutic agents (that is, vincristine) (see Table 5). Below, we review the available data that uniformly supports a role for CB_2 receptor activation in modulation of neuropathic nociception.

Spinal nerve ligation model

The efficacy of CB_2 agonists in suppressing neuropathic nociception was first evaluated using a spinal nerve ligation model (Ibrahim *et al*., 2003). Neuropathic pain was induced by ligating the L5 and L6 spinal nerves according to the procedures described by Kim and Chung (1992). AM1241, administered systemically, produced a dose-dependent reversal of established mechanical and thermal hypersensitivity that was mediated by a CB_2-specific mechanism (Ibrahim *et al*., 2003). The antihyperalgesic effects of AM1241 were reversed by the CB_2 receptor antagonist AM630 (Ibrahim *et al*., 2003). Moreover, AM1241 blocked mechanical and thermal hypersensitivity in both $CB_1^{+/+}$ wild-type and $CB_1^{-/-}$ mice, demonstrating that the antihyperalgesic efficacy of AM1241 does not require activity at CB_1. Another group independently verified that AM1241, administered systemically, dose-dependently suppressed

nerve injury-induced mechanical hypersensitivity on the ligated side compared with vehicle-treated controls; this antihyperalgesic effect was similarly mediated by a CB_2-specific mechanism (Beltramo *et al*., 2006). In this study, L768242 (GW405833) also reduced allodynia elicited by spinal nerve ligation in a dose-dependent manner. However, pharmacological specificity of L768242 (GW405833)-induced actions was not verified using a CB_2 antagonist (Beltramo *et al*., 2006). Additional support for CB_2-mediated suppression of neuropathic nociception is derived from electrophysiological studies employing JWH-133. JWH-133, administered locally in the paw, reduced evoked responses to noxious mechanical stimulation in wide dynamic range neurons recorded in spinal nerve ligated rats; this effect was attenuated by SR144528 (Elmes *et al*., 2004). Moreover, spinal administration of JWH-133 also attenuated the mechanically evoked responses of neuropathic rats in a manner that was blocked by SR144528 (Sagar *et al*., 2005), suggesting that CB_2 agonists may act at central sites to suppress pathological pain states. Responses in sham-operated animals were unaffected by JWH-133 (Sagar *et al*., 2005; but see Elmes *et al*., 2004). Thus, activation of CB_2 receptors with multiple CB_2-selective agonists—AM1241, JWH-133 and L768242 (GW405833)—alleviates neuropathic nociception in behavioural and electrophysiological studies.

Partial sciatic nerve ligation model

Additional support for the hypothesis that CB_2 agonists suppress neuropathic nociception is obtained from studies in which unilateral hindlimb neuropathy was induced by partial sciatic nerve ligation. Partial ligation of the sciatic nerve (Seltzer *et al*., 1990) resulted in the development of tactile allodynia and mechanical hyperalgesia within 2 weeks following surgery. Systemic administration of GW405833 (L768242) 3–5 weeks after the surgery reduced nerve injury-induced mechanical hyperalgesia in rats (Valenzano *et al*., 2005) and mice (Whiteside *et al*., 2005). Interpretation of these studies is somewhat limited by the fact that the pharmacological specificity of GW405833 (L768242) was not assessed in the partial sciatic nerve ligation model. However, the authors did demonstrate that antihyperalgesic effects of the same compound were blocked by a CB_2 antagonist and were absent in $CB_2^{-/-}$ mice following adjuvant inflammation of the hindpaw.

Chemotherapy-induced neuropathy

A single study has evaluated the possible role of CB_2 receptors in suppressing neuropathic nociception evoked by treatment with chemotherapeutic agents (Rahn *et al*., 2007). Unlike neuropathy induced by traumatic nerve injury, neuropathy induced by chemotherapeutic agents may occur in the absence of peripheral nerve degeneration (Polomano and Bennett, 2001). A dysregulation of cellular calcium homeostasis, attributable to atypical mitochondrial function, has been implicated in chemotherapy-evoked neuropathy (Siau and Bennett, 2006). The vinca alkaloid vincristine is a chemotherapeutic agent commonly employed to treat leukaemia, lymphomas and solid tumours (Polomano and Bennett, 2001). Treatment with vincristine induces mechanical allodynia under conditions in which responses to thermal stimulation are preserved (Weng *et al*., 2003; Rahn *et al*., 2007). AM1241 partially reversed vincristine-induced mechanical allodynia in a manner that was blocked by a CB_2 but not a CB_1 antagonist (Rahn *et al*., 2007). By contrast, the mixed cannabinoid agonist WIN55,212-2 fully reversed vincristine-evoked mechanical allodynia. The anti-allodynic effects of WIN55,212-2 were mediated by both CB_1 and CB_2 receptors. Recent work also suggests that CB_2 agonists are effective in suppressing peripheral neuropathy evoked by paclitaxel (taxol) administration in rats (Hohmann *et al*., 2007). More work is necessary to validate the effectiveness of CB_2-selective agonists in suppressing the development of chemotherapy-induced neuropathic pain induced by diverse antitumour agents.

Mechanisms and implications

The complexity of the actions of CB_2 agonists on neuronal and non-neuronal cells and their signalling properties are only beginning to be explored. CB_2 receptors are present at or below the threshold for detection in normal CNS (Munro *et al*., 1993; Griffin *et al*., 1997; Zimmer *et al*., 1999). CB_2 receptors and mRNA have, however, recently been reported within the CNS (Van Sickle *et al*., 2005), including the spinal cord (Beltramo *et al*., 2006), brainstem and cortex (Van Sickle *et al*., 2005). However, CB_2 receptors localized within the CNS are not necessarily associated with neurons. In immunocytochemical studies, definitive evidence for the presence of CB_2 protein within the CNS requires the demonstration that such staining is absent in $CB_2^{-/-}$ mice.

CB_2 receptors have been localized to peripheral nerve terminals (Pertwee *et al*., 1995; Griffin *et al*., 1997). CB_2 receptors were first detected in cultured dorsal root ganglion cells derived from neonatal rats using fluorescence-activated cell sorting analyses (Ross *et al*., 2001). Two structurally distinct CB_2-selective agonists (L768242 (GW405833) and AM1241) have recently been shown to suppress capsaicin-evoked release of calcitonin gene-related peptide in rat spinal cord *in vitro* (Beltramo *et al*., 2006), suggesting a neuronal mechanism of antihyperalgesic action. The presence of CB_2 mRNA and protein has also been reported in rat and mouse paw tissues (Walczak *et al*., 2005, 2006). Finally, CB_2 receptor protein has been identified in microglial cultures of neonatal rat spinal cord (Beltramo *et al*., 2006), suggesting that non-neuronal substrates contribute to the antihyperalgesic actions induced by CB_2-selective agonists *in vivo*. Functional evidence in support of this hypothesis is derived from the ability of the CB_2 agonist JWH-015, administered intrathecally, to reduce paw incision-induced microglial and astrocytic activation in the spinal cord; this reduction was reversed by the CB_2 antagonist AM630 (Romero-Sandoval and Eisenach, 2007). Indeed, activation of CB_2 receptors on non-neuronal cells has been postulated to suppress the release of inflammatory mediators that sensitize nociceptors (Mazzari *et al*., 1996). Thus, non-neuronal substrates as well as neuronal substrates may be responsible for the ability of CB_2-selective agonists to suppress persistent pain states.

These mechanisms may also contribute to the more pronounced effects of selective CB_2 agonists in inflamed compared to noninflamed tissue (Nackley *et al.*, 2004).

Electrophysiological studies demonstrate that CB_2-selective agonists preferentially suppress activity in spinal nociceptive neurons under conditions in which these neurons are sensitized. For example, AM1241 suppresses C-fibre-mediated afterdischarge responses and windup in spinal wide dynamic range neurons through activation of CB_2 receptors (Nackley *et al.*, 2004). This suppression is more pronounced in the presence compared to the absence of persistent inflammation (Nackley *et al.*, 2004). Selective activation of CB_2 receptors by JWH-133 also suppresses mechanically evoked responses in neuropathic but not in sham-operated rats (Elmes *et al.*, 2004; Sagar *et al.*, 2005). JWH-133, administered locally in the paw, also inhibits carrageenan-evoked expansion of peripheral receptive field sizes in WDR neurons (Elmes *et al.*, 2004). These studies collectively suggest that activation of CB_2 receptor mechanisms preferentially suppresses neuronal sensitization. It is thus particularly noteworthy that pathological pain states and injury are associated with upregulation of CB_2 receptor protein and mRNA. Expression of CB_2 is markedly upregulated in dorsal root ganglia and spinal cord following sciatic nerve injury (Zhang *et al.*, 2003; Walczak *et al.*, 2005; Wotherspoon *et al.*, 2005; Beltramo *et al.*, 2006), whereas expression levels remain near the threshold for detection in naive animals. Understanding the functional consequence of upregulation of CB_2 receptors along nociceptive pathways under conditions of pain and injury represents an important direction for future research.

Activation of CB_2 receptors with AM1241 on skin keratinocytes stimulates the production of β-endorphin and induces antinociception in an acute pain model (the plantar test) in otherwise naive animals through activation of μ-opioid receptors (Ibrahim *et al.*, 2005). The extent to which β-endorphin release may contribute to the antihyperalgesic effects of AM1241 in persistent pain state remains to be determined. Antihyperalgesic effects induced by GW405833 (L768242) in the complete Freund's adjuvant model are independent of μ-opioid receptors (Whiteside *et al.*, 2005). This difference in μ-opioid sensitivity between these agonists may account for the ability of AM1241, but not other CB_2 agonists described to date, to induce robust antinociception in the plantar test in otherwise naive animals (see Table 2; but see Bingham *et al.*, 2007). Therefore, it is noteworthy that signalling changes downstream of initial CB_2 receptor activation may differ depending upon the agonist employed and the presence or absence of injury. These factors must be considered in efforts to understand CB_2 agonist actions as well as the antihyperalgesic/antinociceptive phenotype observed in a given nociceptive assay. Further work is required to identify the specific cellular elements that contain CB_2 receptors and mechanism by which activation of these receptors suppresses neuronal sensitization.

Conclusions and limitations

The available data suggest that CB_2-selective agonists show promise for suppressing inflammatory and neuropathic pain states. In animal models of tissue and nerve injury-induced nociception, CB_2-selective agonists suppress hyperalgesia and allodynia and normalize nociceptive thresholds without inducing analgesia. These behavioural observations are also consistent with electrophysiological data demonstrating that CB_2-selective agonists such as AM1241 and JWH-133 suppress responses in nociceptive neurons preferentially under conditions in which these neurons are sensitized (that is, in the presence of pathological pain states). These agonists may also be more efficacious in suppressing hypersensitivity to mechanical as opposed to thermal stimulation for reasons that remain incompletely understood. A particularly beneficial aspect of the pharmacological profile of CB_2 agonists is the failure of these compounds to induce adverse CNS side effects associated with activation of CB_1 receptors. By contrast, unwanted CNS side effects (for example, psychoactivity, hypoactivity and hypothermia) limit the therapeutic potential of mixed cannabinoid agonists that exhibit high affinity for CB_1 receptors. More work is necessary to demonstrate beyond doubt that CB_2-selective agonists are unlikely to be psychoactive or addictive.

The available literature supports the efficacy of CB_2 agonists in suppressing persistent pain states following acute administration. However, the impact of long-term treatment with CB_2 agonists on antihyperalgesic efficacy and immune system function remains largely unknown. Individuals suffering from immunosuppressive diseases (for example, AIDS patients) could be poor candidates for CB_2-mediated pharmacotherapies for pain because of the extensive distribution of CB_2 receptors in immune tissue (for example, mast cells, B cells, microglial cells). More work is needed to identify the limitations associated with therapeutic strategies targeting CB_2 receptors. Further research should also explore the therapeutic potential of multimodal analgesic strategies that combine CB_2-mediated pharmacotherapies for pain with other agents directed at different analgesic targets. Such strategies offer the potential to produce synergistic antihyperalgesic effects with a more beneficial therapeutic ratio compared to conventional analgesics (for example, by combining a CB_2-selective agonist with lower doses of opiates, CB_1 agonists or nonsteroidal anti-inflammatory drugs that are below the threshold for inducing undesirable side effects). More work is necessary to determine whether activation of CB_2 receptors can be employed effectively in chronic pain patients to suppress pathological pain states with limited side effect profiles.

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Conflict of interest

The authors state no conflict of interest.

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