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Cannabinoids and Pain: Sites and Mechanisms of Action

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Abstract

The endocannabinoid system, consisting of the cannabinoid₁ receptor (CB₁R) and cannabinoid₂ receptor (CB₂R), endogenous cannabinoid ligands (endocannabinoids) and metabolising enzymes, is present throughout the pain pathways. Endocannabinoids, phytocannabinoids and synthetic cannabinoid receptor agonists have anti-nociceptive effects in animal models of acute, inflammatory and neuropathic pain. CB₁R and CB₂R are located at peripheral, spinal or supra-spinal sites are important targets mediating these anti-nociceptive effects. The mechanisms underlying the analgesic effects of cannabinoids likely include inhibition of pre-synaptic neurotransmitter and neuropeptide release, modulation of post-synaptic neuronal excitability, activation of the descending inhibitory pain pathway, and reductions in neuroinflammatory signalling. Strategies to dissociate the psychoactive effects of cannabinoids from their analgesic effects have focused on peripherally restricted CB₁R agonists, CB₂R agonists, inhibitors of endocannabinoid catabolism or uptake, and modulation of other non-CB₁R /non-CB₂R targets of cannabinoids including TRPV1, GPR55 and PPARs. The large body of pre-clinical evidence in support of cannabinoids as potential analgesic agents is supported by clinical studies demonstrating their efficacy across a variety of pain disorders.

Keywords: Pain; Cannabinoid receptor; Endocannabinoids; *in vivo*; Pre-clinical; Periphery; Spinal Cord; Brain; Rat; Mouse

List of Abbreviations

2-AG, 2-arachidonyl glycerol

AEA, anandamide

BLA, basolateral nucleus of the amygdala

CB₁R, cannabinoid receptor type 1
CB₂R, cannabinoid receptor type 2
CCI, chronic constriction injury
CeA, central nucleus of the amygdala
CFA, complete Freund's adjuvant
CGRP, calcitonin gene-related peptide
DRG, dorsal root ganglia
FAAH, fatty acid amide hydrolase
GRP55, G protein-coupled receptor 55
I.c.v., intracerebroventricular
I.p., Intraperitoneal
I.t., intrathecal
MAGL, monoacylglycerol lipase
OEA, *N*-oleoylethanolamide
PAG, periaqueductal grey
PEA, *N*-palmitoylethanolamide
PPARs, peroxisome proliferator-activated receptors
PSL, partial sciatic ligation model
RVM, rostral ventromedial medulla
SNI, spared nerve injury
SNL, spinal nerve ligation
TRPV1, Transient Receptor Potential Subfamily V Member 1
 Δ^9 -THC, Δ^9 -tetrahydrocannabinol

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1. Introduction

Cannabis sativa has been used for medicinal purposes, including relief of pain, for thousands of years (Grinspoon & Bakalar, 1993). The isolation and identification of the principal psychoactive constituent of cannabis, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), in the 1960s (Mechoulam & Gaoni, 1967), sparked a search for its mechanism of action which in turn led to the discovery of two cannabinoid receptors, the cannabinoid₁ receptor (CB₁R) (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and cannabinoid₂ receptor (CB₂R) (Munro, Thomas, & Abu-Shaar, 1993). Endogenous ligands (endocannabinoids) which exert their effects upon binding to these cannabinoid receptors were also discovered, the two best characterised being arachidonyl ethanolamide (anandamide; AEA) (Devane et al., 1992) and 2-arachidonyl glycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). The receptors, endocannabinoids, transport proteins and enzymes that synthesise or degrade the endocannabinoids together comprise the endocannabinoid system. A large body of pre-clinical and clinical research indicates that this lipid signalling system modulates a broad range of physiological processes and behaviours including, but not limited to, pain, mood, appetite, emesis, neuronal activity, memory, immunity, cell development and cell fate, and the cardiovascular system. In particular, the anti-nociceptive effects of cannabinoids and endocannabinoid signalling have received a lot of attention over the past 30 years, with thousands of peer-reviewed publications reporting antinociceptive/analgesic effects in preclinical and clinical studies and elucidating the sites and mechanisms of action. The impact of this research has started to be seen in clinical practice with the introduction of the Δ^9 -THC/Cannabidiol buccal spray nabiximols (Sativex®) for the adjunctive treatment of neuropathic pain in multiple sclerosis patients and severe cancer pain in Canada, and with many US states and countries around the world

relaxing their laws to allow patients to use cannabis or cannabinoids for a range of conditions including chronic pain. The present review will focus primarily on the evidence from pre-clinical studies utilising animal models of acute, inflammatory and neuropathic pain with an emphasis on the sites and mechanisms underlying cannabinoid-mediated anti-nociception. For excellent recent reviews and meta-analyses of clinical studies in this area, please see (Barnes, 2006; Boychuk, Goddard, Mauro, & Orellana, 2015; Health, 2016; Iskedjian, Bereza, Gordon, Piwko, & Einarson, 2007; Lynch & Ware, 2015; McCormick et al., 2017; Russo, 2016; Vermersch, 2011; Whiting et al., 2015).

[Insert Table 1 near here]

With regards preclinical studies in rodents, both genetic and pharmacological (Table 1) approaches have been used to demonstrate and understand the modulation of pain by cannabinoids and the endocannabinoid system. Enhanced thermal analgesia and reduced nociceptive behaviour in the formalin and carrageenan models was observed in mice lacking the enzyme fatty acid amide hydrolase (FAAH) which catabolises AEA and other *N*-acylethanolamines including *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA), compared with wild-type controls (Carey et al., 2016; Cravatt et al., 2001; Lichtman, Shelton, Advani, & Cravatt, 2004). These results suggest that one or more FAAH substrates exert antinociceptive actions in these models. FAAH knockout mice, and mice that express FAAH exclusively in nervous tissue, have also been shown to display anti-inflammatory and antihyperalgesic effects in both the carrageenan and collagen-induced arthritis models, effects prevented by administration of a CB₂R, but not CB₁R, antagonist (Kinsey, Naidu, Cravatt, Dudley, & Lichtman, 2011; Lichtman et al., 2004). Thus, the augmented levels of AEA in these mice appears to exert tonic analgesia via CB₂R. However, a pronociceptive phenotype

of FAAH knockout mice can be unmasked following intradermal injection of the TRPV1 agonist capsaicin (Carey et al., 2016). Similarly, mice lacking the 2-AG-catabolising enzyme monoacylglycerol lipase (MAGL) exhibited significantly augmented nociceptive behaviour in the formalin and acetic acid tests and no alterations in thermal tail-withdrawal latency, effects that were likely due to desensitisation of CB₁R (Petrenko, Yamazaki, Sakimura, Kano, & Baba, 2014; Schlosburg et al., 2010). In a recent study, nitroglycerin-induced mechanical allodynia and neuronal activation of the trigeminal nucleus to model migraine were abolished in FAAH-deficient mice, results also seen in mice administered FAAH inhibitors (Nozaki, Markert, & Zimmer, 2015). These effects were shown to be CB₁R-mediated and they infer that one or more FAAH substrates mediate antinociception via CB₁R. Knockouts of the CB₁R have also been generated and exhibit hypoalgesia in the hot plate, tail immersion and formalin tests (Valverde, Karsak, & Zimmer, 2005; Zimmer, Zimmer, Hohmann, Herkenham, & Bonner, 1999), suggesting, somewhat paradoxically, a pro-nociceptive role for CB₁R. However, a different CB₁^{-/-} mouse line displayed similar basal responses to noxious stimuli compared to wild-type animals (Castane et al., 2006; Ledent et al., 1999). Development of mechanical hypersensitivity following partial sciatic nerve ligation was unaltered in CB₁R knockout mice (Castane et al., 2006; Racz, Nent, Erxlebe, & Zimmer, 2015), however these mice did exhibit more pronounced behavioural manifestations of anxiety-related behaviours compared to wild-type mice (Racz et al., 2015), suggesting an anxiolytic role for CB₁R. Mice lacking the CB₂R have also been generated as have CB₁/CB₂ double knockouts (Buckley, 2008; Buckley et al., 2000) and mice overexpressing the CB₂R (La Porta, Bura, Aracil-Fernandez, Manzanares, & Maldonado, 2013). The affective manifestations of osteoarthritis pain in the monosodium iodoacetate model were enhanced in CB₁R knockout mice and absent in CB₂R knockouts, suggesting that the presence of CB₁R attenuates the affective component in this model while CB₂R is

required for expression of the affective component. Both the CB₁R agonist ACEA and the CB₂R agonist JWH133 ameliorated the nociceptive and affective alterations, with ACEA also improving the associated memory impairment (La Porta et al., 2015). It had previously been shown that development of mechanical allodynia in this model was unaltered in CB₁R and CB₂R knockout mice, but attenuated in those overexpressing CB₂R, compared with wild-type mice (La Porta et al., 2013). In another recent study, paclitaxel-induced mechanical and cold allodynia developed to an equivalent degree in mice lacking CB₁R, CB₂R, and wild-type mice (Deng et al., 2015), suggesting that CB₁R and CB₂R do not impact on the development of the pain-related phenotype in this model. Following intraplantar administration of complete Freund's adjuvant (CFA), or partial nerve ligation, mechanical hyperalgesia was absent in mice lacking GPR55 (Staton et al., 2008), a receptor sensitive to some cannabinoids. However, another study reported thermal hyperalgesia in GPR55 knockout mice (Bjursell et al., 2016) and, most recently, it was shown that genetic deletion of GPR55 did not alter the development of pain-related behaviour in a number of mechanistically distinct models of inflammatory and neuropathic pain (Carey et al., 2017).

Shortly after its discovery, Bicher and Mechoulam (1968) showed that Δ^9 -THC was anti-nociceptive in rabbits (Bicher & Mechoulam, 1968). Since then, many studies have shown that cannabinoids are anti-nociceptive following systemic administration (For comprehensive review see Pertwee, 2001). The animal (usually rodent) models used can be divided into three broad groups: (1) acute pain (2) inflammatory pain involving tissue injury (3) neuropathic pain involving peripheral nerve injury. Cannabinoid receptor agonists, administered intraperitoneally, intravenously, subcutaneously or orally, demonstrate analgesic efficacy (to greater or lesser degrees depending on the compound and model under investigation) across these models (Finn & Chapman, 2004; Pertwee, 2001) with a potency

that is comparable with, or greater than, some opiates and cyclooxygenase inhibitors (Bloom & Dewey, 1978; Smith, Cichewicz, Martin, & Welch, 1998; Sofia, Vassar, & Knobloch, 1975; Thorat & Bhargava, 1994). Many of the earlier studies used non-selective cannabinoid receptor agonists, however, the involvement of CB₁ and/or CB₂ receptors has been probed with selective antagonists, and more recent studies have assessed the efficacy of agonists with selectivity for CB₁ or CB₂ receptors. Interpretation of the results of studies employing systemic administration of cannabinoids can, however, sometimes be complicated by cannabinoid-mediated suppression of motor activity. To examine the specific sites and mechanisms through which cannabinoids reduce pain, studies have investigated the anti-nociceptive activity of cannabinoids and endocannabinoid system modulators administered supra-spinally, spinally and peripherally. These site-specific studies are the key focus of the present review.

2. Anatomical localisation of the endocannabinoid system throughout the pain pathway

Two major ascending pain pathways in mammals, the spino-thalamic pathway and the spino-parabrachial pathway, encode the sensory-discriminatory and affective aspects of pain respectively (see Figure 1). In addition, the descending pain pathway originates in higher cortical regions and in the amygdala and hypothalamus, and projects (*via* the periaqueductal grey (PAG)) to the lower brainstem and spinal cord. Descending control of pain can be either inhibitory or facilitatory depending on the precise circuitry and receptors that are engaged (Millan, 2002; Ossipov, Morimura, & Porreca, 2014; Suzuki & Dickenson, 2005; Suzuki, Rygh, & Dickenson, 2004). The endocannabinoid system is expressed throughout the

ascending and descending pain pathways at peripheral, spinal and supra-spinal sites (Figure 1). CB₁ receptors are located on peripheral endings and central terminals of primary afferent neurons (Hohmann, Briley, & Herkenham, 1999; Hohmann & Herkenham, 1998, 1999a). CB₁ receptors are also found in the dorsal root ganglion (DRG) and in the superficial laminae of the spinal cord (Farquhar-Smith et al., 2000; Glass, Dragunow, & Faull, 1997; Herkenham et al., 1991; Hohmann & Herkenham, 1999a; Ross et al., 2001; Sanudo-Pena, Strangman, Mackie, Walker, & Tsou, 1999). Ahluwalia *et al.* (2002) reported that 80% of CB₁R-expressing neurons either contained calcitonin gene-related peptide (CGRP), a marker for peptidergic neurons, or bound IB4, a marker for an unmyelinated neurons which express glycoproteins (Ahluwalia, Urban, Bevan, Capogna, & Nagy, 2002), suggesting a functional role for CB₁R on peripheral nerve terminals. However, there is also evidence that CB₁R mRNA is expressed predominantly in medium and large sized DRG neurons, with lower levels of in DRG neurons expressing substance P or CGRP mRNA (Hohmann & Herkenham, 1999b). In addition to its peripheral and spinal localisation, CB₁R is also located in all of the major brain regions involved in pain processing and modulation. Receptor autoradiography and immunohistochemistry studies have demonstrated the presence of CB₁R in the cortex, amygdala, hypothalamus, thalamus, PAG, parabrachial nucleus (PBN) and in brainstem regions including the rostral ventromedial medulla (RVM) (Glass et al., 1997; Herkenham et al., 1991; Herkenham et al., 1990; Mailleux, Parmentier, & Vanderhaeghen, 1992; Thomas, Wei, & Martin, 1992; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). CB₁R localisation is predominantly presynaptic, and its direct activation by synthetic agonists, or by endocannabinoids that signal retrogradely, inhibits the release of neurotransmitters including GABA and glutamate (Rea, Roche, & Finn, 2007).

[Insert Figure 1 close to here]

The clinical utility of cannabinoids acting at CB₁R can be limited due to adverse central side effects and the development of tolerance (De Vry, Jentzsch, Kuhl, & Eckel, 2004; Gonzalez, Cebeira, & Fernandez-Ruiz, 2005). This has led to increased interest in the role of the CB₂R in pain. The CB₂R has been categorised classically as the peripheral cannabinoid receptor due to its presence on the cells and tissues of the immune, reproductive, cardiovascular, gastrointestinal and respiratory systems and numerous reports which were unable to detect CB₂R transcripts in normal healthy brain (Derbenev, Stuart, & Smith, 2004; Facci et al., 1995; Griffin et al., 1999; Munro et al., 1993). However, more recent evidence suggests that CB₂R are present in the brain under normal and, in particular, under pathological/inflammatory conditions (Baek, Zheng, Darlington, & Smith, 2008; Concannon, Okine, Finn, & Dowd, 2015; Onaivi, Ishiguro, Gong, et al., 2006; M. Roche & Finn, 2010; Van Sickle et al., 2005; H. Y. Zhang et al., 2014), although to a much lesser extent than the ubiquitously expressed CB₁R. CB₂R expression has been demonstrated within pain-related brain regions including the cerebral cortex, hippocampus, striatum, amygdala, thalamic nuclei, PAG, cerebellum and several brain stem nuclei of the rodent brain (Ashton, Friberg, Darlington, & Smith, 2006; Brusco, Tagliaferro, Saez, & Onaivi, 2008; Gong et al., 2006; Onaivi et al., 2008; Onaivi, Ishiguro, Gong, et al., 2006; Onaivi, Ishiguro, Sejal, et al., 2006; Suarez et al., 2008; Van Sickle et al., 2005). Although many studies have identified central CB₂R on glial and endothelial cells, there is also evidence to support the expression of CB₂R on sub-populations of neurons within the central nervous system (Ashton et al., 2006; Beltramo et al., 2006; Gong et al., 2006; Molina-Holgado et al., 2007; Onaivi, Ishiguro, Gong, et al., 2006; Palazuelos et al., 2006; Suarez et al., 2008; Van Sickle et al., 2005; Viscomi et al., 2009; H. Y. Zhang et al., 2014). There is evidence for expression of CB₂R in DRG and in the dorsal horn of the spinal cord and upregulation during neuropathic or

inflammatory pain (Anand et al., 2008; Hsieh et al., 2011; Romero-Sandoval & Eisenach, 2007; Romero-Sandoval, Nutile-McMenemy, & DeLeo, 2008; Ross et al., 2001; Svizenska, Brazda, Klusakova, & Dubovy, 2013; Wotherspoon et al., 2005; J. Zhang et al., 2003). The high expression of the CB₂R in tissues of the immune system including the spleen and thymus as well as on specific immune cells including B lymphocytes, natural killer cells, monocytes, neutrophils and T lymphocytes (Berdyshev, 2000; Howlett et al., 2002; Klein, Newton, & Friedman, 2001; Munro et al., 1993; Sugiura et al., 1995) has focused research on the viability of the CB₂R as a therapeutic target in inflammatory pain conditions in particular, but also neuropathic pain which can have a neuroinflammatory/neuroimmune component (Milligan et al., 2003; Watkins, Milligan, & Maier, 2003).

In addition to the cannabinoid receptors, other components of the endocannabinoid system are also present throughout the ascending and descending pain pathways. Thus, the endocannabinoids, *N*-acylethanolamines and their metabolising enzymes are localised in peripheral tissues innervated by primary afferent nociceptive neurons (Calignano, La Rana, Giuffrida, & Piomelli, 1998; Felder et al., 1996), spinal cord (Di Marzo et al., 2000; Egertova, Giang, Cravatt, & Elphick, 1998; Tsou, Nogueron, et al., 1998), and brain (Devane et al., 1992; Egertova et al., 1998; Hanus et al., 2001; Huang et al., 2002; Porter et al., 2002; Stella, Schweitzer, & Piomelli, 1997; Tsou, Nogueron, et al., 1998) tissues, including regions important in pain. Elegant *in vivo* microdialysis experiments demonstrated that intraplantar injection of the chemical irritant formalin evokes the release of anandamide in the midbrain PAG (Walker, Huang, Strangman, Tsou, & Sanudo-Pena, 1999a). The endocannabinoids and *N*-acylethanolamines also have affinity for, and activity at, a number of non-CB₁/non-CB₂ receptors, including Transient Receptor Potential Subfamily V Member 1 (TRPV1), GPR55 (putative CB₃ receptor) and the peroxisome proliferator-activated receptors (PPARs)

(Alexander & Kendall, 2007; Wiley & Martin, 2002), all of which are also expressed throughout the pain pathways and likely play important roles in endocannabinoid-mediated regulation of pain. The remainder of this review will focus on functional *in vivo* studies of cannabinoids and the endocannabinoid system in models of acute, inflammatory and neuropathic pain with a focus on supraspinal, spinal and peripheral sites and mechanisms of action.

3. Supra-spinal sites and mechanisms of action

3.1 Evidence from acute pain models

In the 1990s, it was demonstrated that the inhibitory effects of the cannabinoid receptor agonists CP-55,940, THC and WIN 55,212-2, administered systemically, on either tail-flick responding (Lichtman & Martin, 1991) or noxious-evoked responses of spinal neurons (Hohmann, Tsou, & Walker, 1999) are abolished in rats following spinal transection, results that suggested an important role for descending inhibitory pathways in mediating cannabinoid-induced anti-nociception. The realisation that CB₁R is present in moderate to high densities in brain regions which play an important role in nociceptive processing (see section 2 above) also prompted investigation of supra-spinal sites of action mediating cannabinoid-induced anti-nociception. Multiple studies have now shown that synthetic or plant-derived cannabinoid receptor agonists, or endogenous cannabinoid ligands, display anti-nociceptive activity in the mouse and rat tail-flick tests, following intracerebroventricular (i.c.v) administration (Fang et al., 2012; Lichtman & Martin, 1997; Martin, Lai, Patrick, Tsou, & Walker, 1993; Pan et al., 2014; Raffa, Stone, & Hipp, 1999; Welch, 1994; Welch, Huffman, & Lowe, 1998; Welch, Thomas, & Patrick, 1995; Zheng et al., 2016).

Significant effort has also been directed at elucidating the specific brain regions that mediate the anti-nociceptive effects of cannabinoid receptor agonists (Corcoran, Roche, & Finn, 2015). In early work by Martin and colleagues, direct administration of WIN55,212-2 into a number of different brain regions including the amygdala, thalamus, superior colliculus and A5 region was shown to be anti-nociceptive in the tail-flick test (Martin et al., 1999). Micro-injection of the non-selective cannabinoid receptor agonists WIN55,212-2 and HU210 into the RVM also elevated tail-flick latencies in rats (Martin, Tsou, & Walker, 1998; Meng & Johansen, 2004). Moreover, the effects of HU210 were attenuated by co-administration with the CB₁R antagonist/inverse agonist rimonabant (Martin et al., 1998). Further evidence for the importance of the RVM in cannabinoid-induced attenuation of acute pain came from a study demonstrating that GABA_A receptor agonist-mediated inactivation of the RVM prevented anti-nociceptive effects of systemically administered WIN55,212-2 in the rat tail-flick test (Meng, Manning, Martin, & Fields, 1998). In addition, it has been shown that the antinociceptive effects of intra-RVM administration of WIN55,212-2 in the tail-flick test are associated with inhibition of ON-cell activity and an increase in OFF-cell activity, effects blocked by rimonabant (Meng & Johansen, 2004). Within the RVM, the nucleus reticularis gigantocellularis pars alpha appears to be an important locus for cannabinoid-mediated antinociception (Monhemius, Azami, Green, & Roberts, 2001).

The PAG, another major component of the descending inhibitory pain pathway, is also an important locus for the anti-nociceptive effects of cannabinoids. Electrical stimulation of the dorsal or lateral columns of the PAG resulted in CB₁R-mediated anti-nociception in the rat tail-flick test which was accompanied by a marked increase in AEA release in the PAG (Walker, Huang, Strangman, Tsou, & Sanudo-Pena, 1999b). Intra-plantar injection of formalin also resulted in increased AEA release, suggesting engagement of an endogenous

cannabinergic pain-modulatory system in this midbrain region. Direct administration of CP55, 940 into the ventrolateral (vl) PAG (Lichtman, Cook, & Martin, 1996) and of WIN55,212-2 into the dorsal PAG (Martin et al., 1999; Martin, Patrick, Coffin, Tsou, & Walker, 1995) had anti-nociceptive effects in the rat tail-flick test. *In vitro* studies of the mechanism of action of cannabinoids at the level of the PAG suggest that cannabinoids reduce neurotransmitter release from pre-synaptic terminals and inhibit GABAergic and glutamatergic transmission (Vaughan, Connor, Bagley, & Christie, 2000). Thus, the anti-nociceptive effects of cannabinoid agonists administered into the PAG may arise from the disinhibition of GABAergic interneurons and the activation of the descending inhibitory controls, with subsequent inhibition of excitatory transmission at the level of the spinal cord. There is also evidence for a CB₁-glutamatergic interaction in the dlPAG in mediating cannabinoid-induced antinociception in the plantar test in rats (Palazzo et al., 2001). The suppression of acute pain (tail-flick response) following exposure to acute stress (footshock) via the phenomenon of stress-induced analgesia has also been shown to be mediated by endocannabinoids acting at CB₁R in the dlPAG and RVM (Hohmann et al., 2005; Suplita, Farthing, Gutierrez, & Hohmann, 2005). There is also evidence that the cannabinoid receptor agonist HU210 can enhance the antinociceptive effects of morphine, and *vice versa*, with a site of action in the vlPAG (Wilson-Poe, Pocius, Herschbach, & Morgan, 2013; Wilson, Maher, & Morgan, 2008). In addition to its activity at cannabinoid receptors, AEA also acts at TRPV1, a receptor that also plays an important role in supraspinal modulation of pain (Madasu, Roche, & Finn, 2015). The TRPV1 agonist capsaicin has been shown to induce initial hyperalgesia in the tail-flick test, followed by antinociception, when injected into the dlPAG (McGaraughty et al., 2003). Similarly, in the rat plantar test, biphasic effects of intra-dlPAG administration of capsaicin have been demonstrated (Palazzo et al., 2002) and intra-vlPAG administration of capsaicin results in glutamate release in the RVM, thereby activating

OFF-cells and producing antinociception (Starowicz et al., 2007). In further work using the rat plantar test, intra-vlPAG injection of a low dose of the FAAH inhibitor URB597 with the CB₁ receptor antagonist/inverse agonist AM251 converted the hyperalgesic effect of low dose URB597 to an antinociceptive effect, while co-administration of URB597 with both the TRPV1 antagonist capsazepine and AM251 abolished all effects (Maione et al., 2006). In comparison, the antinociceptive effect of high dose URB597 was converted to a hyperalgesic effect following TRPV1 antagonism. The URB597-induced antinociceptive effects (TRPV1-mediated) and pronociceptive effects (CB₁ receptor mediated) were associated with enhanced or reduced RVM OFF cell activity, respectively, suggesting URB597-induced modulation of the activity of excitatory PAG output neurons (Maione et al., 2006). Intra-vlPAG injection of the dual FAAH inhibitor and TRPV1 antagonist AA-5-HT increased endocannabinoid levels and had an antinociceptive effect in the rat tail-flick test, with associated inhibition of RVM ON- and OFF-cell activity (de Novellis et al., 2008). These effects were blocked by the CB₁ receptor antagonist AM251 or the TRPV1 antagonist I-RTX and were mimicked by intra-vlPAG co-administration of the FAAH inhibitor URB597 with the TRPV1 antagonist I-RTX (de Novellis et al., 2008). Thus, activity of the descending pain pathway is regulated by the action of endocannabinoids at both CB₁R and TRPV1 in the vlPAG. For an excellent schematic of the possible mechanisms underlying endocannabinoid/endovanilloid-mediated control of nociception in the ventrolateral PAG and RVM see Scheme 1 within Maione et al. (2006). Recently, it has also been shown that intra-PAG administration of the GPR55 agonist lysophosphatidylinositol reduces the nociceptive threshold in the rat hotplate test, an effect blocked upon pretreatment with the GPR55 antagonist ML-193 (Deliu et al., 2015), thereby suggesting a role for this putative CB₃ receptor in the PAG in acute pain processing.

The amygdala is thought to play a role in the affective component of pain and is also a component of the descending pain pathway (Neugebauer, Galhardo, Maione, & Mackey, 2009; Neugebauer, Li, Bird, & Han, 2004). Direct administration of WIN55,212-2 into either the basolateral (BLA) or central (CeA) nucleus of the amygdala has been shown to increase tail-flick latency in rats (Hasanein, Parviz, Keshavarz, & Javanmardi, 2007; Martin et al., 1999). Intra-CeA, but not intra-BLA, administration of muscimol, significantly attenuated the anti-nociceptive effects of systemically administered WIN55,212-2 in rats (Manning, Martin, & Meng, 2003). Another study from the same group found that the amygdala also plays a role in cannabinoid-induced antinociception in non-human primates (Manning, Merin, Meng, & Amaral, 2001). Pharmacological blockade of CB₁R in the rat BLA attenuated the stress-induced suppression of nociceptive responding in the tail-flick test (Connell, Bolton, Olsen, Piomelli, & Hohmann, 2006). A role for CB₁R signalling in the rat prelimbic cortex in facilitation of stress-induced analgesia has also been demonstrated (Freitas, Salgado-Rohner, Hallak, Crippa, & Coimbra, 2013). Using fMRI, it has been shown that THC reduces the reported unpleasantness, but not the intensity of ongoing pain and hyperalgesia, induced by capsaicin in healthy human subjects, an effect positively correlated with amygdala activity. THC also reduced functional connectivity between the amygdala and primary sensorimotor areas during the ongoing-pain state (Lee et al., 2013).

3.2 Evidence from inflammatory pain models

Some studies have investigated the effects of intra-cerebral administration of cannabinoids specifically in animal models of inflammatory pain. Direct micro-injection of WIN55,212-2 into the nucleus reticularis gigantocellularis pars alpha, a major source of descending modulation, reduced formalin-evoked pain behaviour, via the CB₁ receptor (Monhemius, Azami, Green, & Roberts, 2001). Administration of the potent cannabinoid receptor agonist

HU210 into the dorsal PAG inhibited formalin-evoked nociceptive behaviour during the second phase and was anti-aversive in rats (Finn et al., 2004; Finn et al., 2003). Intra-vIPAG administration of AA-5-HT to rats prevented the changes in ON- and OFF-cell firing activity induced by intra-plantar injection of formalin, and reversed the formalin-induced increase in locus coeruleus adrenergic cell activity (de Novellis et al., 2008). Injection of the CB₁ receptor antagonist AM251 into the PAG or RVM reverses metazinol-induced analgesia in the rat carrageenan model of inflammatory pain, suggesting a role for the endocannabinoid system in these brain regions in NSAID-induced analgesia (Escobar et al., 2012). These data provide additional evidence that the RVM and PAG are important brain regions mediating the anti-nociceptive effects of cannabinoids in animal models of inflammatory pain. Evidence that pharmacological blockade of CB₁R in the dIPAG attenuates conditioned fear-induced suppression of formalin-evoked nociceptive behaviour (i.e. fear-conditioned analgesia) further substantiates the key role of the endocannabinoid system in the PAG in stress-induced analgesia (Olango, Roche, Ford, Harhen, & Finn, 2012). Conversely, anxiety and depression may exacerbate pain and are frequently found co-morbid with chronic pain. Finn and co-workers have demonstrated that hyperalgesia to intra-plantar formalin injection in Wistar-Kyoto rats that exhibit an anxiodepressive phenotype (versus Sprague-Dawley counterparts) is associated with impaired endocannabinoid-CB₁R signalling in the RVM (Rea et al., 2014). Recently, it has been shown that while CB₁R-mediated inhibition of GABAergic neurons in the RVM is reduced in the rat CFA model, CB₂R functionality in this region is increased in this model of persistent inflammatory pain (Li, Suchland, & Ingram, 2017), supporting the contention that CB₂R may represent a viable analgesic target.

Unilateral inactivation of the CeA reduced the suppression of formalin-evoked c-Fos expression by WIN55,212-2 in the superficial dorsal horn of the spinal cord (Manning et al.,

2003). Furthermore, intra-BLA administration of WIN55,212-2 has also been shown to reduce formalin-evoked nociceptive behaviour in rats, an effect attenuated by intra-BLA administration of the CB₁R antagonist AM251 (Hasanein et al., 2007). Interestingly, intra-BLA administration of rimonabant has also been reported to attenuate formalin-evoked nociceptive behaviour and associated increases in c-Fos immunoreactivity in the hippocampus and RVM in rats (Roche et al., 2010; Roche, O'Connor, Diskin, & Finn, 2007), although intra-BLA administration of AM251 did not have this effect (Rea et al., 2013). In contrast, intra-BLA administration of AM251 (Rea et al., 2013), but not rimonabant (Roche et al., 2010; Roche et al., 2007), attenuated fear-conditioned analgesia in rats. The same doses of rimonabant and AM251 were microinjected into the BLA in these studies and under very similar methodological conditions. However, as discussed in Rea et al. (2013), discrepancies between the effects of the two CB₁R antagonists/inverse agonist may relate to dose–response differences between the 2 compounds when administered into this brain region or to differential activity of the two compounds at non-CB₁R targets expressed in the BLA (e.g. GPR55, TRPV1 or PPARs). There is also evidence that fear-conditioned analgesia is mediated by endocannabinoid-CB₁R signalling in the ventral hippocampus (Ford, Kieran, Dolan, Harhen, & Finn, 2011).

In the rat kaolin/carrageenan intra-articular injection model of arthritis, co-activation of mGluR5 and CB₁R increased activity of prefrontal cortex neurons and inhibited pain-related neuronal activity in the CeA (Ji & Neugebauer, 2014). Further evidence for a role of the endocannabinoid system in the prefrontal cortex in arthritic conditions comes from work demonstrating that osteoarthritis pain is associated with increased 2-AG levels in the prefrontal cortex of mice in the monosodium iodacetate model (La Porta, et al., 2015). Recently, Finn and co-workers demonstrated that the antinociceptive effects of *N*-

palmitoylethanolamide injected into the anterior cingulate cortex in the rat formalin test are likely mediated by AEA-induced activation of CB₁R in this brain region arising from substrate competition between PEA and AEA at FAAH (Okine et al., 2016). A facilitatory role for PPARs and TRPV1 in the anterior cingulate cortex in formalin-evoked nociceptive behaviour has also been suggested (Okine et al., 2016; Okine et al., 2014).

3.3 Evidence from neuropathic pain models

Increased levels of AEA and 2-AG, have been reported in the PAG and RVM of rats 7 days post chronic constriction injury (CCI) of the sciatic nerve, when hyperalgesia and mechanical allodynia were observed to be maximal (Petrosino et al., 2007). Partial sciatic nerve injury has been shown to reduce formalin-evoked pain behaviour in rats (Monhemius et al., 2001). This effect was blocked by direct administration of rimonabant into the nucleus reticularis gigantocellularis pars, suggesting that increased endocannabinoid tone in neuropathic rats can modulate nociceptive behaviour (Monhemius et al., 2001). In the thalamus, CB₁R mRNA is up-regulated in a rat model of neuropathic pain (Siegling, Hofmann, Denzer, Mauler, & De Vry, 2001). Potentially, up-regulation of thalamic CB₁R in neuropathic pain states may serve to enhance the analgesic effects of cannabinoids under these conditions. Interestingly, it has been shown that CB₂R plays a functional role in the modulation of responses of neurons in the ventral posterior nucleus of the thalamus in spinal nerve ligated, but not sham-operated, rats (Jhaveri et al., 2008).

TRPV1 expression is increased in glutamatergic neurons of the medial prefrontal cortex following spared nerve injury (SNI) in rats (Giordano et al., 2012). Moreover, SNI-induced neuropathic pain is also associated with increased levels of endovanilloids and endocannabinoids in the medial prefrontal cortex and direct administration of AA-5-HT into

the prelimbic and infralimbic cortices reduces nociceptive behaviour in rats following SNI (de Novellis et al., 2011; Giordano et al., 2012).

4. Spinal sites and mechanisms of action

4.1 Evidence from acute pain models

Early evidence that the synthetic cannabinoid levonantradol produced a dose-dependent increase in the hot plate and tail-flick response latencies following intrathecal (i.t.) administration (Yaksh, 1981), followed by studies elucidating mechanisms of THC-induced analgesia (Smith & Martin, 1992), indicated a spinal component in the antinociceptive action of the cannabinoids. Behavioural (Smith & Martin, 1992; Yaksh, 1981), electrophysiological (Hohmann, Tsou, & Michael Walker, 1998; Johanek, Simone, & Lisa, 2005; Sokal, Elmes, Kendall, & Chapman, 2003) and neurochemical (Hohmann, Tsou, & Walker, 1999) studies have demonstrated that cannabinoids act at the spinal level to suppress nociceptive processing. In a model of tonic pain, immunocytochemistry for the protooncogene *c-fos* (a marker for the activation of nociceptive neurons in the spinal cord) was used to demonstrate that cannabinoids reduce behavioral responses to noxious stimuli by decreasing spinal processing of nociceptive inputs (Tsou, Martin, & Bereiter, 1996).

4.2 Evidence from inflammatory pain models

The CB₁R has been suggested to be tonically active in the spinal cord under normal conditions and its activity is increased in response to injections of CFA in the plantar surface of the rat hindpaw (Martin, Loo, & Basbaum, 1999). The synthetic mixed CB₁R/CB₂R agonist WIN55212-2 reverses inflammation-induced allodynia at doses that do not produce

analgesia; additionally SR141716A differentially affects the pattern of Fos expression in the spinal cord, depending on the presence or absence of inflammation (Martin et al., 1999).

A functional inhibitory effect of i.t administration of the CB₂R-selective agonists A-836339 and AM1241 have been demonstrated in CFA-induced chronic inflammatory pain (Hsieh et al., 2011). These data complement the findings that CB₂R mRNA is up-regulated in the spinal cord only from rats under inflammatory conditions, suggesting that CB₂R agonists may elicit analgesic effects by acting not only at peripheral DRG sites but also at central levels of the spinal cord, making CB₂ an attractive target for chronic pain treatment, avoiding the adverse psychotropic effects that can accompany CB₁R-based therapies. The antinociceptive effects of A-836339 were not sensitive to pre-treatment with naloxone, and thus are not mediated by μ -opioid receptors. Interestingly, the blockade of AM1241 by naloxone was observed in the CFA model of inflammatory pain (Hsieh et al., 2011).

4.3 Evidence from neuropathic pain models

Cannabinoids suppress C-fiber-evoked responses of dorsal horn neurons recorded in a rat model of neuropathic pain (Elmes et al., 2004). The synaptic processes that produce “wind-up”, the phenomenon whereby repeated stimulation of cutaneous C-fibres at frequencies > 0.3 Hz gives increasing responses of dorsal horn cells and withdrawal reflexes, are sufficient to produce central sensitization, which appears to be an important component of hyperalgesia and allodynia. The effect of cannabinoids, namely of the potent, synthetic cannabinoid receptor agonist WIN 55,212-2 on wind-up of spinal dorsal horn neurons was investigated in 1999 (Strangman, Walker, & Strangman, 1999). Strangman and Walker provided the first direct evidence that cannabinoids inhibit the activity-dependent facilitation of spinal nociceptive responses. These authors suggested that cannabinoids may act as

general inhibitors of central sensitization by inhibiting calcium entry (Strangman et al., 1999).

The effectiveness of cannabinoids is inconsistent in preclinical neuropathic pain models. WIN 55,212-2 delivered i.t. is effective in mitigating mechanical allodynia in the CCI model (Lim, Sung, Ji, & Mao, 2003) while Costa et al. (Barbara Costa et al., 2005) demonstrated that systemic administration of a CB₁R antagonist significantly reduces mechanical and thermal hyperalgesia in CCI rats and in mice. Others (Toniolo et al., 2014) (Ueda et al., 2014) have also suggested that CB₁R expression and activation can be maladaptive. Very recent research indicates that CB₁R expression contributes to the development of persistent mechanical hypersensitivity, protects against the development of cold allodynia but is not involved in motor impairment following spared-nerve injury in mice (Sideris et al., 2016).

Although nerve injury increased CB₂R expression in spinal microglia (Zhang et al., 2003), CB₂R agonists suppressed microglial activation and reduced neuropathic pain symptoms (Wilkerson et al., 2012). I.t. delivery of the CB₂R agonist JWH-015 reverses hypersensitivity following nerve injury in a CB₂R- and not CB₁R-dependent manner (an effect blocked by AM630 but not AM281) (Romero-Sandoval & Eisenach, 2007). Interestingly, CB₂R knockout mice displayed increased microglial and astrocytic reactivity in the spinal cord and enhanced neuropathic pain symptoms, whereas transgenic mice overexpressing CB₂R showed attenuated glial reactivity and neuropathic pain (Racz et al., 2008). CB₂R are up-regulated on both microglia and astrocytes following spared nerve injury in mice, and chronic systemic administration of the CB₂R agonist NESS400 reduces pain behaviour, astrogliosis, microglial activation, and levels of proinflammatory cytokines, whilst promoting levels of anti-inflammatory cytokines (Luongo et al., 2010).

Interestingly, the CB₂-selective agonists A-836339 and AM1241, which have previously been shown to counteract inflammatory pain, have also been proven to alleviate neuropathic pain in the rat SNL model (Hsieh et al., 2011). As in the case of CFA-induced inflammation, A-836339 action was opioid-insensitive, while the blockade of AM1241 by naloxone was not observed. The reason for the difference between two drugs is currently unknown. AM1241 may interact with additional targets that may contribute to the antinociceptive efficacy through the regulation of the opioid receptor pathway (Hsieh et al., 2011). However, there is some conflicting evidence in the literature, with a recent study reporting no effect of the CB₂ agonists GW405833 and JWH-133 on mechanical allodynia in CCI model of neuropathy (Brownjohn & Ashton, 2012). This study also reported no elevation of CB₂ at either the protein or mRNA level, probably due to the choice of neuropathic pain model (SNL or CCI).

The endocannabinoids AEA and 2-AG are also increased in the spinal cord following induction of a neuropathic pain state in a CCI model (Petrosino et al., 2007; Starowicz et al., 2012), suggesting that pharmacological manipulation of endocannabinoid accumulation or breakdown may suppress neuropathic nociception in rodents. Both FAAH and MAGL represent potential therapeutic targets for the development of pharmacological agents to treat chronic pain resulting from nerve injury. A significant reduction of neuropathic pain symptoms following inhibition of the AEA hydrolytic enzyme with URB597 in a rat CCI model was reported (Starowicz et al., 2012, 2013). Depending on the dose of URB597 used, and on the consequent lesser or higher elevation of endogenous AEA levels, analgesia was mediated via CB₁ or TRPV1 receptors, respectively. These data suggest that indirect modulation of TRPV1 function, as well as strengthening endogenous AEA signalling by inhibiting its enzymatic degradation, together hold promise for the development of novel

multi-target pharmacological treatments. These studies highlight the importance of the endocannabinoid system as a potential therapeutic target for treatment of neuropathic pain.

5. Peripheral sites and mechanisms of action

5.1 Evidence from acute pain models

In behavioural experiments, administration of the endogenous CB₁R agonist, AEA, into the ipsilateral hindpaw of the rat reduced formalin-induced nociception (Calignano, La Rana, Giuffrida, Piomelli, 1998), indicating that activation of peripheral CB₁R produces antinociception. PEA produced a similar effect by activating peripheral CB₂R. Furthermore, PEA was administered together with AEA, the two compounds acted synergistically. The peripheral actions of CB₁R agonists are attributed to an inhibition of both the sensitizing effects of NGF and CGRP release (Richardson, Kilo, & Hargreaves, 1998) and (Rice, Farquhar-Smith, & Nagy, 2002) .

In 2001, it was demonstrated that selective activation of peripheral CB₂R results in antinociception (Malan, Ibrahim, Deng, Liu, Mata, Vanderah, Porreca, 2001). AM1241, the CB₂R-selective agonist, administered both locally and systemically (i.p.) produced thermal hypoalgesia, which was absent when the compound was coadministered with AM630, a CB₂R antagonist, but not AM251, the CB₁R antagonist. AM1241 administered locally to the contralateral paw did not elicit antinociception, which suggests a local site of action. Moreover, local administration of AM630 blocked the antinociceptive effect of AM1241 injected i.p., further implicating peripheral CB₂R as the main site of action. Ibrahim et al. (Ibrahim, Porreca, Lai, Albrecht, Rice, Khodorova, Davar, Makriyannis, Vanderah, Mata,

Malan, 2005) reported that CB₂R activation produces antinociception by stimulating the release of β -endorphin from keratinocytes, which in turn acts at μ -opioid receptors on primary afferent neurons. Furthermore, it was also suggested that other mediators might be released from local cells after activation of CB₂R, contributing to its antinociceptive effects. Nonetheless, β -endorphin release was suggested to be critical for CB₂R-mediated antinociception because the effects of AM1241 were completely prevented by a β -endorphin-sequestering antiserum (Ibrahim, Porreca, Lai, Albrecht, Rice, Khodorova, Davar, Makriyannis, Vanderah, Mata, Malan, 2005).

Inhibition of endocannabinoid metabolism is considered a promising therapeutic target on its own. It has been demonstrated that blocking AEA degradation results in antinociceptive effects in the mouse hotplate test (Kathuria et al., 2003). The carbamate compound URB597 reduces pain-related behaviour in the rat produced by prior i.pl. injection of CFA in a manner blocked by a CB₁R but not a CB₂R antagonist (Wilson et al., 2005). Also global deletion of FAAH results in lower inflammatory response to local administration of carrageenan (Lichtman, Shelton, Advani, & Cravatt, 2004). There is good evidence in the literature that CB₂R may regulate oedema and hyperalgesia in response to carrageenan (Holt, Comelli, Costa, & Fowler, 2005). Antioedemic effect of the CB₂R agonists, AM1241 and JTE-907, was demonstrated (Quartilho et al., 2003) (Iwamura, Suzuki, Ueda, Kaya, & Inaba, 2001). Moreover, URB597 reduced oedema formation in a CB₂R-dependent manner (Holt et al., 2005).

5.2 Evidence from inflammatory pain models

Studies have demonstrated that administration of the endogenous CB₁R agonist, AEA into the ipsilateral hindpaw of the rat reduces carrageenan-induced hyperalgesia (Richardson

et al., 1998) and that administration of the PEA reduced oedema and inflammatory hyperalgesia (Mazzari, Canella, Petrelli, Marcolongo, & Leon, 1996). It was demonstrated that activation of CB₂R suppresses the development of inflammatory pain (Nackley, Makriyannis, & Hohmann, 2003). AM1241, when injected i.p. suppressed the development of carrageenan-evoked thermal and mechanical hyperalgesia as well as allodynia in a CB₂-dependent manner. Furthermore intraplantar administration suppressed hyperalgesia and allodynia only on the inflamed paw and was inactive following administration in the contralateral (noninflamed) paw (Nackley et al., 2003).

As a result of systemic administration of the selective FAAH inhibitor, URB597, elevation in endogenous AEA levels reduced the mechanical allodynia and thermal hyperalgesia in an inflammatory pain model in both CB₁R- and CB₂R-dependent manner (Jayamanne et al., 2006). Moreover, two distinct inhibitors of MAGL (JZL184 and URB602) elicited local analgesia in the formalin-induced pain model that involved both CB₁R and CB₂R. URB602 produced regionally restricted increases in 2-AG levels in rat hind paw skin without altering AEA levels (Guindon, Guijarro, Piomelli, & Hohmann, 2011). The above findings indicate that increase in endocannabinoid tone block the development of inflammatory pain.

5.3 Evidence from neuropathic pain models

Studies by Fox et al., 2001 (Fox et al., 2001) and Elmes (Elmes, Jhaveri, Smart, Kendall, & Chapman, 2004) showed that antinociceptive effects in the PSL and SNL models were produced by the activation of peripheral CB₁R and CB₂R, respectively. In particular, WIN 55,212-2 reversed mechanical hyperalgesia following intraplantar administration into the ipsilateral hind paw (Fox et al., 2001). CB₁ mRNA is localized in DRG neurons and CB₁R has been shown to undergo peripheral axonal flow in the sciatic nerve (Hohmann &

Herkenham, 1999). Moreover, data from Hargreaves' group indicate that CB₁R activation inhibits sensory neuropeptide release from the skin of rat hindpaws, demonstrating a functional inhibitory activity on peripheral sensory nerves (Richardson et al., 1998). JWH-133, a cannabinoid CB₂R agonist, also significantly reduced noxious mechanically evoked responses of wide dynamic range dorsal horn neurons following intra-plantar injections (Elmes et al., 2004). Indeed CB₂ agonists offer promise in neuropathic pain management. CCI of the sciatic nerve induced neuropathic pain behaviour and bilateral elevation of both CB₂R protein and mRNA in lumbar L4-L5 as well as cervical C7-C8 DRG when compared with naive animals. CB₂R protein and mRNA were increased not only in DRG neurons but also in satellite glial cells. Such changes suggest propagation of neuroinflammation alongside the neuraxis and the neuroprotective effects of CB₂R (Svízenská, Brázda, Klusáková, & Dubový, 2013). Work of Leichsenring et al. analysed the effect of repeated i.p. administration of the CB₂R agonist GW-405,833 on mechanical allodynia, compared with the potent cannabinoid receptor agonist WIN-55,212-2 (Leichsenring, Andriske, Bäcker, Stichel, & Lübbert, 2009). Both drugs, applied daily at a low non-psychotropic dose, were equally effective in reducing mechanical allodynia induced by SNL. A reappearance of glial activation was also associated with return of neuropathic pain-related behavior in this study (Leichsenring, Andriske, Bäcker, Stichel, & Lübbert, 2009). The involvement of peripheral CB₂R in neuropathic pain symptoms alleviation was also a subject of studies by Kinsey (Kinsey et al., 2011). An ethyl sulfonamide THC analogue, O-3223, a selective CB₂ agonist, was reported to reduce thermal hyperalgesia in the CCI-induced neuropathic pain model. Its anti-hyperalgesic effects were blocked by pretreatment with the CB₂R selective antagonist SR144528, but not by the CB₁R antagonist, rimonabant. In addition, O-3223 (unlike CP-55,940, CB₁R and CB₂R agonist), did not elicit hypothermia or motor disturbances,

indicating it has significant anti-inflammatory and anti-nociceptive effects *in vivo*, but does not cause CB₁R-mediated side-effects.

The therapeutic utility of locally administered AEA for neuropathic pain was proven by Guindon and Beaulieu (Guindon & Beaulieu, 2006). However surprising data on the lack of anti-allodynic and anti-hyperalgesic effects of URB597 in a neuropathic pain model were published by (Jayamanne et al. 2006). In animals subjected to partial ligation of the sciatic nerve, i.p. administration of the selective FAAH inhibitor, URB597, produced no significant change in mechanical paw withdrawal latency. It has been suggested that repeated administration of URB597 may prove to be more efficacious in neuropathic pain models, as observed previously for exogenous cannabinoid receptor agonists (Costa et al., 2004). Moreover, acute administration of the irreversible FAAH inhibitor, URB597 and of the reversible FAAH inhibitor, OL-135, decreases allodynia in mouse CCI model of neuropathic pain (Kinsey et al., 2009). This attenuation was completely blocked by pre-treatment with either CB₁- or CB₂R antagonists. Given the neuroinflammatory nature of the nerve injury in the CCI model, it is not surprising that both cannabinoid receptors play a role in modulating neuropathic pain.

Another FAAH inhibitor, PF-3845, characterized by an increased FAAH specificity and longer duration of *in vivo* activity (Kinsey, Long, Cravatt, & Lichtman, 2010) also showed an attenuation of CCI-induced mechanical and cold allodynia in wildtype mice (Kinsey et al., 2009). Subsequent work from the Lichtman group explored the contribution of CB₁R and/or CB₂R for the antiallodynic effects of the FAAH and the MAGL inhibitors in a mouse model of neuropathic pain (Kinsey et al., 2010) and further confirmed that both CB₁ and CB₂R are necessary for the antiallodynic effects of FAAH inhibitors, while only CB₁R are necessary

for the antiallodynic effects caused by MAGL inhibition. These data indicate that the endocannabinoids may affect different levels of the nociceptive and inflammatory pathways involved in neuropathic pain.

6. Conclusions and future directions

Cannabinoids exert a direct antinociceptive effect on pain of different origins. The CB₁R-mediated analgesic effects of cannabinoid ligands are well established, but limited by their side-effect profile. The observation that CB₂R activation produces desirable actions in a range of preclinical models (Leleu-Chavain et al., 2012) (Han, Thatte, Buzard, & Jones, 2013) attracted considerable interest. However, despite very favorable efficacy in a range of preclinical models, CB₂ agonists have fared poorly in the clinic (Dhopeshwarkar & Mackie, 2014). The targeted manipulation of the endocannabinoid system might also be beneficial in the face of inflammation and chronic pain conditions. Interestingly investigations into the endocannabinoids and their effector sites, along with other non-cannabinoid receptors, have exploded in recent years, and insights reveal this area of pharmacology to be highly complex and dynamic (Piscitelli & Di Marzo, 2012) (Starowicz & Di Marzo, 2013). Data derived from complex and clinically relevant animal models highlights the question of effectiveness of dual-acting compounds (Ligresti et al., 2014)(Aiello, Carullo, Badolato, & Brizzi, 2016) (Malek & Starowicz, 2016) and support the case for multi-target pharmacological intervention for effective pain treatment.

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Figure Legend

Figure 1. Cannabinoid receptor distribution throughout the pain pathways. Cannabinoid receptors are present at all three levels of pain processing: **A) in the periphery:** CB₁R are present in the peripheral sensory nerve endings, both CB₁R and CB₂R are expressed in the dorsal root ganglion (DRG); **B) in the spinal cord:** CB₁R are found in the dorsolateral funiculus, in the surroundings of the central canal and in the superficial dorsal horn. CB₂R are expressed on glial cells highly restricted to lumbar spinal cord; its expression coincides with the appearance of activated microglia and **C) in the supra-spinal sites:** CB₁R are distributed in areas of the brain involved in pain processing, perception and modulation eg. thalamus, amygdala, parabrachial nucleus, periaqueductal grey matter and rostroventral medulla. They are also present in caudate nucleus and putamen (n. accumbens), basal ganglia, hypothalamus and cerebellum. CB₂R receptor are expressed in some neurons within the brainstem, and also on glial cells in the cerebellum and cortex. CB₁R and CB₂R distribution in regions involved in pain transduction, transmission, perception and modulation provides the anatomical basis for the well-known ability of CB₁/CB₂R agonists to decrease pain.

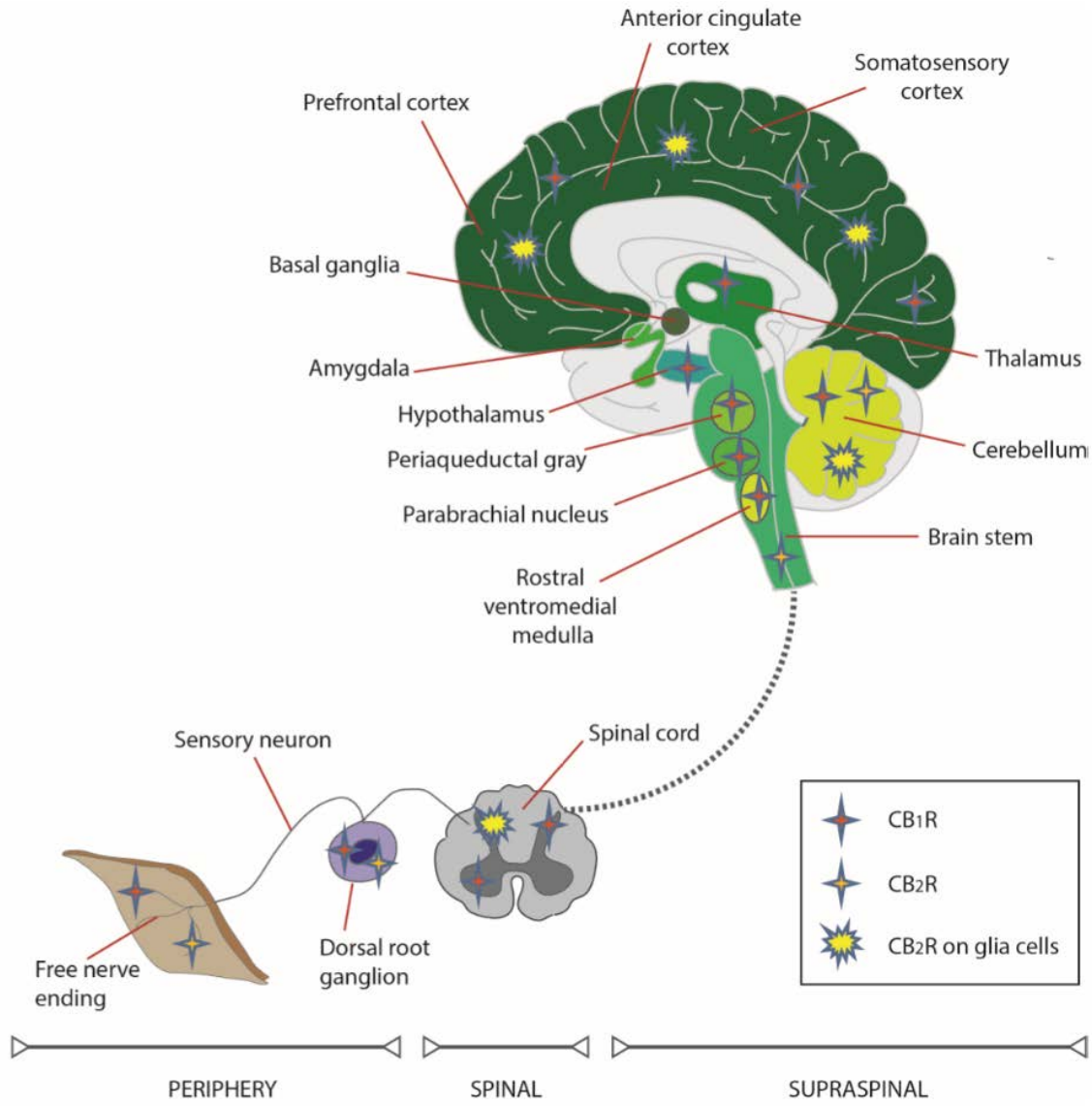


Table 1. Compounds, referred to in the text, and used to elucidate the role of cannabinoids and the endocannabinoid system in pain modulation.

Pharmacological substance	IUPAC name	Target
THC	(-)-(6a <i>R</i> ,10a <i>R</i>)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6 <i>H</i> -benzo[<i>c</i>]chromen-1-ol; Δ ⁹ -tetrahydrocannabinol	The main psychotropic constituent of cannabis, CB ₁ /CB ₂ receptor partial agonist
ACEA	<i>N</i> -(2-Chloroethyl)-5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i> -eicosatetraenamide	Potent and highly selective synthetic CB ₁ receptor agonist, has low affinity for CB ₂
2-AG AEA	(5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i>)-5,8,11,14-Eicosatetraenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 2-Arachidonoyl glycerol <i>N</i> -(2-Hydroxyethyl)-5 <i>Z</i> ,8 <i>Z</i> ,11 <i>Z</i> ,14 <i>Z</i> -eicosatetraenamide; anandamide	Endogenous CB ₁ and CB ₂ receptor agonists without any marked selectivity for either sub-type. AEA is also an agonist at TRPV1
WIN 55,212-2 CP-55,940 HU-210	{(R)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3- <i>de</i>]-1,4-benzoxazin-6-yl](1-naphthyl)methanone } {(-)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol } 3-(1,1'-dimethylheptyl)-6a <i>R</i> ,7,10,10a <i>R</i> -tetrahydro-1-hydroxy-6,6-dimethyl-6 <i>H</i> -dibenzo[<i>b,d</i>]pyran-9-methanol	Mixed CB ₁ /CB ₂ receptor agonist
AM251	<i>N</i> -(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 <i>H</i> -pyrazole-3-carboxamide	CB ₁ -selective antagonists

AM281	1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl- <i>N</i> -4-morpholinyl-1 <i>H</i> -pyrazole-3-carboxamide	
SR141716A (rimonabant)	[<i>N</i> -(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 <i>H</i> -pyrazole-3-carboxamide hydrochloride] (rimonabant)	
A-836339	[<i>N</i> (<i>Z</i>)]- <i>N</i> -[3-(2-methoxyethyl)-4,5-dimethyl-2(3 <i>H</i>)-thiazolylidene]-2,2,3,3-tetramethyl-cyclopropanecarboxamide	CB ₂ -selective agonists
AM1241	(2-iodo-5-nitrophenyl)-(1-(1-methylpiperidin-2-ylmethyl)-1 <i>H</i> -indol-3-yl)methanone	
GW-405,833	1-(2,3-Dichlorobenzoyl)-5-methoxy-2-methyl-3-[2-(4-morpholinyl)ethyl]-1 <i>H</i> -indole	
JTE-907	<i>N</i> -(1,3-benzodioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentoxo-1 <i>H</i> -quinoline-3-carboxamide	
JWH-015	(2-methyl-1-propylindol-3-yl)-naphthalen-1-ylmethanone	
JWH-133	(6a <i>R</i> ,10a <i>R</i>)-6,6,9-trimethyl-3-(2-methylpentan-2-yl)-6a,7,10,10a-tetrahydrobenzo[<i>c</i>]chromene	
NESS400	1-(2',4'-dichlorophenyl)-6-methyl- <i>N</i> -cyclohexylamine-1,4-dihydroindeno[1,2- <i>c</i>]pyrazole-3-carboxamide	
O-3223	(6a <i>R</i> ,10a <i>R</i>)-6,6,9-trimethyl-3-(2-methylpentan-2-yl)-6a,7,10,10a-tetrahydrobenzo[<i>c</i>]chromene	
AM630	6-iodopravadoline	CB ₂ -selective antagonists

SR144528	5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1R,3S,4S)-2,2,4-trimethyl-3-bicyclo[2.2.1]heptanyl]pyrazole-3-carboxamide	
OL-135	7-phenyl-1-(5-pyridin-2-yl-1,3-oxazol-2-yl)heptan-1-one	FAAH inhibitor
PF-3845	N-pyridin-3-yl-4-[[3-[5-(trifluoromethyl)pyridin-2-yl]oxyphenyl]methyl]piperidine-1-carboxamide	
URB597	(3-phenylphenyl) N-(4-methoxyphenyl)carbamate	
JZL184	4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate	MAGL inhibitor
URB602	[1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester	
I-RTX	6,7-Deepoxy-6,7-didehydro-5-deoxy-21-dephenyl-21-(phenylmethyl)-daphnetoxin,20-(4-hydroxy-5-iodo-3-methoxybenzeneacetate); Iodoresiniferatoxin	Potent TRPV1 antagonist
AA-5-HT	(5Z,8Z,11Z,14Z)-N-(2-(5-hydroxy-1 <i>H</i> -indol-3-yl)ethyl)icosa-5,8,11,14-tetraenamide; N-arachidonoyl-serotonin	FAAH inhibitor and TRPV1 antagonist
ML-193 (alternative name CID 1261822)	N-[4-[[3,4-Dimethyl-5-isoxazolyl]amino]sulfonyl]phenyl]-6,8-dimethyl-2-(2-pyridinyl)-4-quinolinecarboxamide	Potent and selective GPR55 antagonist