CHAPTER THIRTEEN

Cannabinoids and Pain: Sites and Mechanisms of Action

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Abstract

The endocannabinoid system, consisting of the cannabinoid$_1$ receptor (CB_1R) and cannabinoid$_2$ receptor (CB_2R), endogenous cannabinoid ligands (endocannabinoids), and metabolizing enzymes, is present throughout the pain pathways. Endocannabinoids, phytocannabinoids, and synthetic cannabinoid receptor agonists have antinociceptive effects in animal models of acute, inflammatory, and neuropathic pain. CB_1R and CB_2R located at peripheral, spinal, or supraspinal sites are important targets mediating these antinociceptive effects. The mechanisms underlying the analgesic effects of

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cannabinoids likely include inhibition of presynaptic neurotransmitter and neuropeptide release, modulation of postsynaptic neuronal excitability, activation of the descending inhibitory pain pathway, and reductions in neuroinflammatory signaling. Strategies to dissociate the psychoactive effects of cannabinoids from their analgesic effects have focused on peripherally restricted CB_1R agonists, CB_2R agonists, inhibitors of endocannabinoid catabolism or uptake, and modulation of other non-CB_1R/non-CB_2R targets of cannabinoids including TRPV1, GPR55, and PPARs. The large body of preclinical evidence in support of cannabinoids as potential analgesic agents is supported by clinical studies demonstrating their efficacy across a variety of pain disorders.

ABBREVIATIONS

2-AG 2-arachidonoyl glycerol
AEA anandamide
BLA basolateral nucleus of the amygdala
CB_1R cannabinoid receptor type 1
CB_2R 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
GPR55 G protein-coupled receptor 55
i.p. intraperitoneal
i.t. intrathecal
MAGL monoacylglycerol lipase
PAG periaqueductal gray
PEA *N*-palmitoylethanolamide
PPARs peroxisome proliferator-activated receptors
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

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 cannabinoid$_1$ receptor (CB_1R) (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and cannabinoid$_2$ receptor (CB_2R) (Munro, Thomas, & Abu-Shaar, 1993). Endogenous ligands (endocannabinoids) which exert their effects upon binding to these cannabinoid receptors were also discovered, the two best characterized being arachidonoyl ethanolamide (anandamide, AEA) (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). The receptors, endocannabinoids, transport proteins, and enzymes that synthesize or degrade the endocannabinoids together comprise the endocannabinoid system. A large body of preclinical and clinical research indicates that this lipid signaling system modulates a broad range of physiological processes and behaviors 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 antinociceptive effects of cannabinoids and endocannabinoid signaling 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 preclinical studies utilizing animal models of acute, inflammatory, and neuropathic pain with an emphasis on the sites and mechanisms underlying cannabinoid-mediated antinociception. For excellent recent reviews and meta-analyses of clinical studies in this area, please see Barnes (2006), Boychuk, Goddard, Mauro, and Orellana (2015), Canadian Agency for Drugs and Technologies in Health (2016), Iskedjian, Bereza, Gordon, Piwko, and Einarson (2007), Lynch and Ware (2015), McCormick et al. (2017), Russo (2016), Vermersch (2011), and Whiting et al. (2015).

With regards preclinical studies in rodents, both genetic and pharmacological (Table 1) approaches have been used to demonstrate and understand

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	(−)-(6*aR*,10*aR*)-6,6,9-Trimethyl-3-pentyl-6*a*,7,8,10*a*-tetrahydro-6*H*-benzo[*c*]chromen-1-ol; Δ^9-tetrahydrocannabinol	The main psychotropic constituent of cannabis, CB_1/CB_2 receptor partial agonist
ACEA	*N*-(2-Chloroethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide	Potent and highly selective synthetic CB_1 receptor agonist has low affinity for CB_2
2-AG	(5*Z*,8*Z*,11*Z*,14*Z*)-5,8,11,14-Eicosatetraenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 2-arachidonoyl glycerol	Endogenous CB_1 and CB_2 receptor agonists without any marked selectivity for either subtype. AEA is also an agonist at TRPV1
AEA	*N*-(2-Hydroxyethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide; anandamide	
WIN55,212-2	{(*R*)-(+)-[2,3-Dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone}	Mixed CB_1/CB_2 receptor agonist
CP-55,940	{(−)-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol}	
HU210	3-(1,1′-Dimethylheptyl)-6*aR*,7,10,10*aR*-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol	
AM251	*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide	CB_1-selective antagonists
AM281	1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide	
SR141716A (rimonabant)	[*N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] (rimonabant)	

A-836339	[*N*(*Z*)]-*N*-[3-(2-Methoxyethyl)-4,5-dimethyl-2(3*H*)-thiazolylidene]-2,2,3,3-tetramethyl-cyclopropanecarboxamide	CB_2-selective agonists
AM1241	(2-Iodo-5-nitrophenyl)-(1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl)methanone	
GW-405,833	1-(2,3-Dichlorobenzoyl)-5-methoxy-2-methyl-3-[2-(4-morpholinyl) ethyl]-1*H*-indole	
JTE-907	*N*-(1,3-Benzodioxol-5-ylmethyl)-7-methoxy-2-oxo-8-pentoxy-1*H*-quinoline-3-carboxamide	
JWH-015	(2-Methyl-1-propylindol-3-yl)-naphthalen-1-ylmethanone	
JWH-133	(6*aR*,10*aR*)-6,6,9-Trimethyl-3-(2-methylpentan-2-yl)-6*a*,7,10,10*a*-tetrahydrobenzo[*c*]chromene	
NESS400	1-(2′,4′-Dichlorophenyl)-6-methyl-*N*-cyclohexylamine-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide	
O-3223	(6*aR*,10*aR*)-6,6,9-Trimethyl-3-(2-methylpentan-2-yl)-6*a*,7,10,10*a*-tetrahydrobenzo[*c*]chromene	
AM630	6-Iodopravadoline	CB_2-selective antagonists
SR144528	5-(4-Chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*R*,3*S*,4*S*)-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	

Continued

Table 1 Compounds Referred to in the Text and Used to Elucidate the Role of Cannabinoids and the Endocannabinoid System in Pain Modulation—cont'd

Pharmacological Substance	IUPAC Name	Target
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	(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(2-(5-Hydroxy-1*H*-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

the modulation of pain by cannabinoids and the endocannabinoid system. Enhanced thermal analgesia and reduced nociceptive behavior in the formalin and carrageenan models were observed in mice lacking the enzyme fatty acid amide hydrolase (FAAH) which catabolizes AEA and other *N*-acylethanolamines including *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide, 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 antiinflammatory and antihyperalgesic effects in both the carrageenan and collagen-induced arthritis models, effects prevented by administration of a CB_2R, but not CB_1R, antagonist (Kinsey, Naidu, Cravatt, Dudley, & Lichtman, 2011; Lichtman et al., 2004). Thus, the augmented levels of AEA in these mice appear to exert tonic analgesia via CB_2R. However, a pronociceptive phenotype of FAAH knockout mice can be unmasked following intradermal injection of the transient receptor potential subfamily V member 1 (TRPV1) agonist capsaicin (Carey et al., 2016). Similarly, mice lacking the 2-AG-catabolizing enzyme monoacylglycerol lipase (MAGL) exhibited significantly augmented nociceptive behavior in the formalin and acetic acid tests and no alterations in thermal tail-withdrawal latency, effects that were likely due to desensitization of CB_1R (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 CB_1R mediated and they infer that one or more FAAH substrates mediate antinociception via CB_1R. Knockouts of the CB_1R 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 pronociceptive role for CB_1R. However, a different $CB_1^{-/-}$ 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 CB_1R knockout mice (Castane et al., 2006; Racz, Nent, Erxlebe, & Zimmer, 2015); however, these mice did exhibit more pronounced behavioral manifestations of anxiety-related behaviors compared to wild-type

mice (Racz et al., 2015), suggesting an anxiolytic role for CB_1R. Mice lacking the CB_2R have also been generated as have CB_1/CB_2 double knockouts (Buckley, 2008; Buckley et al., 2000) and mice overexpressing the CB_2R (La Porta, Bura, Aracil-Fernandez, Manzanares, & Maldonado, 2013). The affective manifestations of osteoarthritis pain in the monosodium iodoacetate model were enhanced in CB_1R knockout mice and absent in CB_2R knockouts, suggesting that the presence of CB_1R attenuates the affective component in this model, while CB_2R is required for expression of the affective component. Both the CB_1R agonist ACEA and the CB_2R agonist JWH-133 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 CB_1R and CB_2R knockout mice, but attenuated in those overexpressing CB_2R, 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 CB_1R, CB_2R, and wild-type mice (Deng et al., 2015), suggesting that CB_1R and CB_2R 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 behavior 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 antinociceptive in rabbits (Bicher & Mechoulam, 1968). Since then, many studies have shown that cannabinoids are antinociceptive 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, and (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 nonselective cannabinoid receptor agonists; however, the involvement of CB_1 and/or CB_2 receptors has been probed with selective antagonists, and more recent studies have assessed the efficacy of agonists with selectivity for CB_1 or CB_2 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 antinociceptive activity of cannabinoids and endocannabinoid system modulators administered supraspinally, spinally, and peripherally. These site-specific studies are the key focus of the present review.

2. ANATOMICAL LOCALIZATION OF THE ENDOCANNABINOID SYSTEM THROUGHOUT THE PAIN PATHWAY

Two major ascending pain pathways in mammals, the spinothalamic pathway and the spinoparabrachial pathway, encode the sensory-discriminatory and affective aspects of pain, respectively (see Fig. 1). In addition, the descending pain pathway originates in higher cortical regions and in the amygdala and hypothalamus, and projects (via the periaqueductal gray (PAG)) to the lower brain stem 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 supraspinal sites (Fig. 1). CB_1 receptors are located on peripheral endings and central terminals of primary afferent neurons (Hohmann, Briley, & Herkenham, 1999; Hohmann & Herkenham, 1998, 1999a). CB_1 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, 1999b; Ross et al., 2001; Sanudo-Pena, Strangman, Mackie, Walker, & Tsou, 1999). Ahluwalia, Urban, Bevan, Capogna, and Nagy (2002) reported that 80% of CB_1R-expressing neurons either contained calcitonin gene-related peptide (CGRP), a marker for peptidergic neurons, or bound IB4, a marker

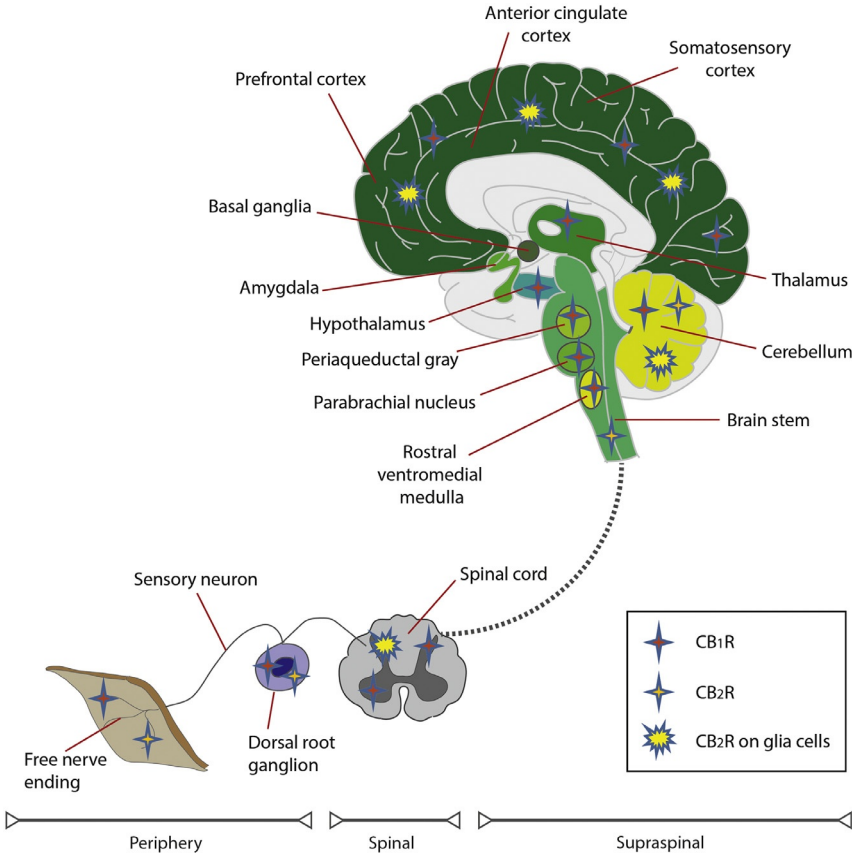

Fig. 1 Cannabinoid receptor distribution throughout the pain pathways. Cannabinoid receptors are present at all three levels of pain processing: (A) in the periphery: CB_1R is present in the peripheral sensory nerve endings, both CB_1R and CB_2R are expressed in the dorsal root ganglion (DRG); (B) in the spinal cord: CB_1R is found in the dorsolateral funiculus, in the surroundings of the central canal, and in the superficial dorsal horn. CB_2R is expressed on glial cells highly restricted to lumbar spinal cord; its expression coincides with the appearance of activated microglia, and (C) in the supraspinal sites: CB_1R is distributed in areas of the brain involved in pain processing, perception, and modulation, e.g., thalamus, amygdala, parabrachial nucleus, periaqueductal gray matter, and rostroventral medulla. They are also present in caudate nucleus and putamen (n. accumbens), basal ganglia, hypothalamus, and cerebellum. CB_2R is expressed in some neurons within the brain stem, and also on glial cells in the cerebellum and cortex. CB_1R and CB_2R distribution in regions involved in pain transduction, transmission, perception, and modulation provides the anatomical basis for the well-known ability of CB_1/CB_2R agonists to decrease pain.

for an unmyelinated neurons which express glycoproteins (Ahluwalia et al., 2002), suggesting a functional role for CB_1R on peripheral nerve terminals. However, there is also evidence that CB_1R mRNA is expressed predominantly in medium- and large-sized DRG neurons, with lower levels in DRG neurons expressing substance P or CGRP mRNA (Hohmann & Herkenham, 1999b). In addition to its peripheral and spinal localization, CB_1R 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 CB_1R in the cortex, amygdala, hypothalamus, thalamus, PAG, parabrachial nucleus, and in brain stem regions including the rostral ventromedial medulla (RVM) (Glass et al., 1997; Herkenham et al., 1991, 1990; Mailleux, Parmentier, & Vanderhaeghen, 1992; Thomas, Wei, & Martin, 1992; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). CB_1R localization 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).

The clinical utility of cannabinoids acting at CB_1R 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 CB_2R in pain. The CB_2R has been categorized 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 CB_2R 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 CB_2R is 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; Roche & Finn, 2010; Van Sickle et al., 2005; Zhang et al., 2014), although to a much lesser extent than the ubiquitously expressed CB_1R. CB_2R 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 CB_2R on glial and endothelial cells, there is also evidence to support the expression of CB_2R on subpopulations 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; Zhang et al., 2014). There is evidence for expression of CB_2R 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; Zhang et al., 2003). The high expression of the CB_2R 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 CB_2R 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 metabolizing enzymes are localized 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 AEA in the midbrain PAG (Walker, Huang, Strangman, Tsou, & Sanudo-Pena, 1999). The endocannabinoids and *N*-acylethanolamines also have affinity for, and activity at, a number of non-CB_1/non-CB_2 receptors, including TRPV1, GPR55 (putative CB_3 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. SUPRASPINAL 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 WIN55,212-2, administered systemically, on either tail-flick responding (Lichtman & Martin, 1991) or noxious-evoked responses of spinal neurons (Hohmann, Tsou, & Walker, 1999a) are abolished in rats following spinal transection, results that suggested an important role for descending inhibitory pathways in mediating cannabinoid-induced antinociception. The realization that CB_1R is present in moderate to high densities in brain regions which play an important role in nociceptive processing (see Section 2) also prompted investigation of supraspinal sites of action mediating cannabinoid-induced antinociception. Multiple studies have now shown that synthetic or plant-derived cannabinoid receptor agonists, or endogenous cannabinoid ligands, display antinociceptive activity in the mouse and rat tail-flick tests, following intracerebroventricular 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., 2017).

Significant effort has also been directed at elucidating the specific brain regions that mediate the antinociceptive 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 antinociceptive in the tail-flick test (Martin, Coffin, et al., 1999). Microinjection of the nonselective 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 CB_1R 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 antinociceptive 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 antinociceptive effects of cannabinoids. Electrical stimulation of the dorsal or lateral columns of the PAG resulted in CB_1R-mediated antinociception in the rat tail-flick test which was accompanied by a marked increase in AEA release in the PAG (Walker et al. 1999). Intraplantar 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 CP-55,940 into the ventrolateral (vl) PAG (Lichtman, Cook, & Martin, 1996) and of WIN55,212-2 into the dorsal (dl) PAG (Martin, Coffin, et al., 1999; Martin, Patrick, Coffin, Tsou, & Walker, 1995) had antinociceptive 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 presynaptic terminals and inhibit GABAergic and glutamatergic transmission (Vaughan, Connor, Bagley, & Christie, 2000). Thus, the antinociceptive 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 CB_1–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 CB_1R 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, Maher, & Morgan, 2008;

Wilson-Poe, Pocius, Herschbach, & Morgan, 2013). 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 CB_1 receptor antagonist/inverse agonist AM251 converted the hyperalgesic effect of low dose URB597 to an antinociceptive effect, while coadministration 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 (CB_1 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 CB_1 receptor antagonist AM251 or the TRPV1 antagonist I-RTX and were mimicked by intra-vlPAG coadministration 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 CB_1R 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 hot plate test, an effect blocked upon pretreatment with the GPR55 antagonist ML-193 (Deliu et al., 2015), thereby suggesting a role for this putative CB_3 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, Coffin, et al., 1999). Intra-CeA, but not intra-BLA, administration of muscimol, significantly attenuated the antinociceptive 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 nonhuman primates (Manning, Merin, Meng, & Amaral, 2001). Pharmacological blockade of CB_1R 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 CB_1R signaling 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 intracerebral administration of cannabinoids specifically in animal models of inflammatory pain. Direct microinjection of WIN55,212-2 into the nucleus reticularis gigantocellularis pars alpha, a major source of descending modulation, reduced formalin-evoked pain behavior, via the CB_1 receptor (Monhemius et al., 2001). Administration of the potent cannabinoid receptor agonist HU210 into the dlPAG inhibited formalin-evoked nociceptive behavior during the second phase and was antiaversive in rats (Finn et al., 2004, 2003). Intra-vlPAG administration of AA-5-HT to rats prevented the changes in ON- and OFF-cell firing activity induced by intraplantar injection of formalin and reversed the formalin-induced increase in locus coeruleus adrenergic cell activity (de Novellis et al., 2008). Injection of the CB_1 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 antinociceptive effects of cannabinoids in animal models of inflammatory pain. Evidence that pharmacological blockade of CB_1R in the dlPAG attenuates conditioned fear-induced suppression of formalin-evoked nociceptive behavior (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 comorbid with chronic pain. Finn and coworkers have demonstrated that hyperalgesia to intraplantar formalin injection in Wistar–Kyoto rats that exhibit an anxiodepressive phenotype (vs Sprague–Dawley counterparts) is associated with impaired endocannabinoid–CB_1R signaling in the RVM (Rea et al., 2014). Recently, it has been shown that while CB_1R-mediated inhibition of GABAergic neurons in the RVM is reduced in the rat CFA model, CB_2R functionality in this region is increased in this model of persistent inflammatory pain (Li, Suchland, & Ingram, 2017), supporting the contention that CB_2R 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 behavior in rats, an effect attenuated by intra-BLA administration of the CB_1R antagonist AM251 (Hasanein et al., 2007). Interestingly, intra-BLA administration of rimonabant has also been reported to attenuate formalin-evoked nociceptive behavior and associated increases in c-Fos immunoreactivity in the hippocampus and RVM in rats (Roche, O'Connor, Diskin, & Finn, 2007; Roche et al., 2010), 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, 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 CB_1R antagonists/inverse agonist may relate to dose–response differences between the two compounds when administered into this brain region or to differential activity of the two compounds at non-CB_1R targets expressed in the BLA (e.g., GPR55, TRPV1, or

PPARs). There is also evidence that fear-conditioned analgesia is mediated by endocannabinoid–CB_1R signaling in the ventral hippocampus (Ford, Kieran, Dolan, Harhen, & Finn, 2011).

In the rat kaolin/carrageenan intraarticular injection model of arthritis, coactivation of mGluR5 and CB_1R 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 iodoacetate model (La Porta et al., 2015). Recently, Finn and coworkers demonstrated that the antinociceptive effects of PEA injected into the anterior cingulate cortex in the rat formalin test are likely mediated by AEA-induced activation of CB_1R 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 behavior has also been suggested (Okine et al., 2016, 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 postchronic 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 behavior 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 behavior (Monhemius et al., 2001). In the thalamus, CB_1R mRNA is upregulated in a rat model of neuropathic pain (Siegling, Hofmann, Denzer, Mauler, & De Vry, 2001). Potentially, upregulation of thalamic CB_1R in neuropathic pain states may serve to enhance the analgesic effects of cannabinoids under these conditions. Interestingly, it has been shown that CB_2R 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 behavior 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. Behavioral (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, 1999a, 1999b) 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 CB_1R 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 hind paw (Martin, Loo, & Basbaum, 1999). The synthetic mixed CB_1R/CB_2R agonist WIN55,212-2 reverses inflammation-induced allodynia at doses that do not produce analgesia; additionally rimonabant differentially affects the pattern of Fos expression in the spinal cord, depending on the presence or absence of inflammation (Martin, Coffin, et al., 1999).

A functional inhibitory effect of i.t. administration of the CB_2R-selective agonists A-836339 and AM1241 has been demonstrated in CFA-induced chronic inflammatory pain (Hsieh et al., 2011). These data complement the findings that CB_2R mRNA is upregulated in the spinal cord only from rats under inflammatory conditions, suggesting that

CB_2R agonists may elicit analgesic effects by acting not only at peripheral DRG sites but also at central levels of the spinal cord, making CB_2 an attractive target for chronic pain treatment, avoiding the adverse psychotropic effects that can accompany CB_1R-based therapies. The antinociceptive effects of A-836339 were not sensitive to pretreatment 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, Jhaveri, Smart, Kendall, & Chapman, 2004). The synaptic processes that produce "windup," the phenomenon whereby repeated stimulation of cutaneous C-fibers 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 WIN55,212-2 on windup 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. WIN55,212-2 delivered i.t. is effective in mitigating mechanical allodynia in the CCI model (Lim, Sung, Ji, & Mao, 2003), while Costa et al. (2005) demonstrated that systemic administration of a CB_1R 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 CB_1R expression and activation can be maladaptive. Very recent research indicates that CB_1R expression contributes to the development of persistent mechanical hypersensitivity, protects against the development of cold allodynia, but is not involved in motor impairment following SNI in mice (Sideris et al., 2016).

Although nerve injury increased CB_2R expression in spinal microglia (Zhang et al., 2003), CB_2R agonists suppressed microglial activation and reduced neuropathic pain symptoms (Wilkerson et al., 2012). I.t. delivery

of the CB_2R agonist JWH-015 reverses hypersensitivity following nerve injury in a CB_2R- and not CB_1R-dependent manner (an effect blocked by AM630 but not AM281) (Romero-Sandoval & Eisenach, 2007). Interestingly, CB_2R knockout mice displayed increased microglial and astrocytic reactivity in the spinal cord and enhanced neuropathic pain symptoms, whereas transgenic mice overexpressing CB_2R showed attenuated glial reactivity and neuropathic pain (Racz et al., 2008). CB_2R is upregulated on both microglia and astrocytes following SNI in mice, and chronic systemic administration of the CB_2R agonist NESS400 reduces pain behavior, astrogliosis, microglial activation, and levels of proinflammatory cytokines, while promoting levels of antiinflammatory cytokines (Luongo et al., 2010).

Interestingly, the CB_2-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 spinal nerve ligation (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 CB_2 agonists GW-405,833 and JWH-133 on mechanical allodynia in CCI model of neuropathy (Brownjohn & Ashton, 2012). This study also reported no elevation of CB_2 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., 2013, 2012). Depending on the dose of URB597 used, and on the consequent lesser or higher elevation of endogenous AEA levels, analgesia was mediated via CB_1 or TRPV1 receptors, respectively. These data suggest that indirect modulation of TRPV1 function, as well as strengthening endogenous AEA signaling by inhibiting its enzymatic degradation, together

hold promise for the development of novel multitarget 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 behavioral experiments, administration of the endogenous CB_1R agonist, AEA, into the ipsilateral hind paw of the rat reduced formalin-induced nociception (Calignano et al., 1998), indicating that activation of peripheral CB_1R produces antinociception. PEA produced a similar effect by activating peripheral CB_2R. Furthermore, PEA was administered together with AEA, the two compounds acted synergistically. The peripheral actions of CB_1R agonists are attributed to an inhibition of both the sensitizing effects of NGF and CGRP release (Rice, Farquhar-Smith, & Nagy, 2002; Richardson, Kilo, & Hargreaves, 1998).

In 2001, it was demonstrated that selective activation of peripheral CB_2R results in antinociception (Malan et al., 2001). AM1241, the CB_2R-selective agonist, administered both locally and systematically (i.p.) produced thermal hypoalgesia, which was absent when the compound was coadministered with AM630, a CB_2R antagonist, but not AM251, the CB_1R 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 CB_2R as the main site of action. Ibrahim et al. (2005) reported that CB_2R 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 CB_2R, contributing to its antinociceptive effects. Nonetheless, β-endorphin release was suggested to be critical for CB_2R-mediated antinociception because the effects of AM1241 were completely prevented by a β-endorphin-sequestering antiserum (Ibrahim et al., 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 hot plate test (Kathuria et al., 2003). The carbamate compound URB597 reduces pain-related behavior in the rat produced by prior i.p. injection of CFA in a manner blocked by a CB_1R but not a CB_2R antagonist (Wilson,

Clayton, Medhurst, Bountra, & Chessell, 2004). Also global deletion of FAAH results in lower inflammatory response to local administration of carrageenan (Lichtman et al., 2004). There is good evidence in the literature that CB_2R may regulate oedema and hyperalgesia in response to carrageenan (Holt, Comelli, Costa, & Fowler, 2005). Antioedemic effect of the CB_2R agonists, AM1241 and JTE-907, was demonstrated (Iwamura, Suzuki, Ueda, Kaya, & Inaba, 2001; Quartilho et al., 2003). Moreover, URB597 reduced oedema formation in a CB_2R-dependent manner (Holt et al., 2005).

5.2 Evidence From Inflammatory Pain Models

Studies have demonstrated that administration of the endogenous CB_1R agonist, AEA, into the ipsilateral hind paw 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 CB_2R 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 CB_2-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 CB_1R- and CB_2R-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 CB_1R and CB_2R. URB602 produced regionally restricted increases in 2-AG levels in rat hind paw skin without altering AEA levels (Guindon, Guijarro, Piomelli, & Hohmann, 2011). The earlier findings indicate that increase in endocannabinoid tone blocks the development of inflammatory pain.

5.3 Evidence From Neuropathic Pain Models

Studies by Fox et al. (2001) and Elmes et al. (2004) showed that antinociceptive effects in the partial sciatic nerve ligation and SNL models were produced by the activation of peripheral CB_1R and CB_2R, respectively.

In particular, WIN55,212-2 reversed mechanical hyperalgesia following intraplantar administration into the ipsilateral hind paw (Fox et al., 2001). CB_1 mRNA is localized in DRG neurons, and CB_1R has been shown to undergo peripheral axonal flow in the sciatic nerve (Hohmann & Herkenham, 1999a, 1999b). Moreover, data form Hargreaves' group indicate that CB_1R activation inhibits sensory neuropeptide release from the skin of rat hind paws, demonstrating a functional inhibitory activity on peripheral sensory nerves (Richardson et al., 1998). JWH-133, a cannabinoid CB_2R agonist, also significantly reduced noxious mechanically evoked responses of wide dynamic range dorsal horn neurons following intraplantar injections (Elmes et al., 2004). Indeed CB_2 agonists offer promise in neuropathic pain management. CCI of the sciatic nerve-induced neuropathic pain behavior and bilateral elevation of both CB_2R protein and mRNA in lumbar L4–L5 as well as cervical C7–C8 DRG when compared with naive animals. CB_2R 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 CB_2R (Svizenska et al., 2013). Work of Leichsenring et al. analyzed the effect of repeated i.p. administration of the CB_2R agonist GW-405,833 on mechanical allodynia, compared with the potent cannabinoid receptor agonist WIN55,212-2 (Leichsenring, Andriske, Bäcker, Stichel, & Lübbert, 2009). Both drugs, applied daily at a low nonpsychotropic 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 et al., 2009). The involvement of peripheral CB_2R in neuropathic pain symptoms alleviation was also a subject of studies by Kinsey, Mahadevan, et al. (2011) and Kinsey, Naidu, et al. (2011). An ethyl sulfonamide THC analogue, O-3223, a selective CB_2 agonist, was reported to reduce thermal hyperalgesia in the CCI-induced neuropathic pain model. Its antihyperalgesic effects were blocked by pretreatment with the CB_2R-selective antagonist SR144528, but not by the CB_1R antagonist, rimonabant. In addition, O-3223 (unlike CP-55,940, CB_1R and CB_2R agonist) did not elicit hypothermia or motor disturbances, indicating it has significant antiinflammatory and antinociceptive effects in vivo, but does not cause CB_1R-mediated side effects.

The therapeutic utility of locally administered AEA for neuropathic pain was proven by Guindon and Beaulieu (2006). However, surprising data on the lack of antiallodynic and antihyperalgesic 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 pretreatment with either CB_1 or CB_2R 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 wild-type mice (Kinsey et al., 2009). Subsequent work from the Lichtman group explored the contribution of CB_1R and/or CB_2R 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 CB_1 and CB_2R are necessary for the antiallodynic effects of FAAH inhibitors, while only CB_1R is 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. CONCLUSION

Cannabinoids exert a direct antinociceptive effect on pain of different origins. The CB_1R-mediated analgesic effects of cannabinoid ligands are well established, but limited by their side-effect profile. The observation that CB_2R activation produces desirable actions in a range of preclinical models (Han, Thatte, Buzard, & Jones, 2013; Leleu-Chavain et al., 2012) attracted considerable interest. However, despite very favorable efficacy in a range of preclinical models, CB_2 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 noncannabinoid 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 highlight the question of effectiveness of dual-acting compounds (Aiello, Carullo, Badolato, & Brizzi, 2016; Ligresti et al., 2014; Malek & Starowicz, 2016) and support the case for multitarget pharmacological intervention for effective pain treatment.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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