

The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain

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A great need exists for the development of new medications to treat pain resulting from various disease states and types of injury. Given that the endogenous cannabinoid (that is, endocannabinoid) system modulates neuronal and immune cell function, both of which play key roles in pain, therapeutics targeting this system hold promise as novel analgesics. Potential therapeutic targets include the cannabinoid receptors, type 1 and 2, as well as biosynthetic and catabolic enzymes of the endocannabinoids *N*-arachidonylethanolamine and 2-arachidonoylglycerol. Notably, cannabinoid receptor agonists as well as inhibitors of endocannabinoid-regulating enzymes fatty acid amide hydrolase and monoacylglycerol lipase produce reliable antinociceptive effects, and offer opioid-sparing antinociceptive effects in myriad preclinical inflammatory and neuropathic pain models. Emerging clinical studies show that ‘medicinal’ cannabis or cannabinoid-based medications relieve pain in human diseases such as cancer, multiple sclerosis, and fibromyalgia. However, clinical data have yet to demonstrate the analgesic efficacy of inhibitors of endocannabinoid-regulating enzymes. Likewise, the question of whether pharmacotherapies aimed at the endocannabinoid system promote opioid-sparing effects in the treatment of pain reflects an important area of research. Here we examine the preclinical and clinical evidence of various endocannabinoid system targets as potential therapeutic strategies for inflammatory and neuropathic pain conditions.

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INTRODUCTION

Chronic pain, such as inflammatory or neuropathic pain, represents a complicated condition that not only diminishes quality of life but also comes at great economic cost. The mechanisms of pathological pain are complex and characterized by both peripheral and central neuronal alterations and neuroimmune activation, which modulates in the initiation and maintenance of chronic pain. Glial cells, including microglia, astrocytes, and oligodendrocytes, located within the central nervous system (CNS), as well as Schwann cells located in the peripheral nervous system (PNS), modulate inflammation after nerve injury (Machelska and Celik, 2016). Following injury, neuronal inflammation and reparatory mechanisms of neural tissues induce a state of peripheral hyperexcitability in primary afferent nociceptors. Additionally, these peripheral nociceptors synapse with

neurons found within the dorsal horn of the spinal cord that undergo dramatic functional alterations, due to the loss of activity of inhibitory neurons. When this loop persists in an enduring way, it results in a persistent pain, which is often resistant to treatments. Indeed, the lack of efficacy of conventional pharmacotherapies to reduce pain, and the significant side effects associated with available medications, creates an unmet need, which has fueled drug discovery efforts for novel analgesics.

A large body of preclinical research demonstrates the effectiveness of cannabinoids in rodent models of acute and chronic inflammatory pain, as well as neuropathic pain (Richardson, 2000). In comparison, few published studies have examined the antinociceptive effects of cannabinoids in nonhuman primates. In the rhesus monkey warm water tail withdrawal paradigm, the endogenous cannabinoid arachidonylethanolamine (anandamide; AEA), the primary active constituent of cannabis Δ^9 -tetrahydrocannabinol (THC), and the high efficacy synthetic cannabinoid receptor agonist WIN55,212-2 produce dose-dependent antinociceptive effects (Manning *et al*, 2001; Vivian *et al*, 1998). Other studies also demonstrate that THC and another high efficacy

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cannabinoid receptor agonist, CP55,940, evoke antinociceptive effects in rhesus monkeys (Li *et al*, 2008; Maguire and France, 2014, 2016).

Several clinical studies have demonstrated the analgesic effects of cannabinoids in human disease states, including pain associated with diabetes, chemotherapy, multiple sclerosis, and fibromyalgia (Lynch and Ware, 2015; Whiting *et al*, 2015). However, cannabinoids lack efficacy for acute pain (for example, dental pain, postoperative pain, and so on (Stevens and Higgins, 2017)). The primary psychoactive constituent of cannabis, THC (Mechoulam and Gaoni, 1965), and certain other plant-derived or synthetic psychoactive cannabinoids bind cannabinoid (CB₁ and CB₂) receptors (Devane *et al*, 1988; Matsuda *et al*, 1990; Munro *et al*, 1993). These cannabinoid receptors belong to the G protein-coupled superfamily and are heterogeneously distributed throughout the CNS and PNS. CB₁ receptors are highly expressed on presynaptic neurons in the brain (Tsou *et al*, 1998), spinal cord (Farquhar-Smith *et al*, 2000), and dorsal root ganglia (Hohmann and Herkenham, 1999; Sañudo-Peña *et al*, 1999). In contrast, CB₂ receptors are primarily expressed in immune cells, including myeloid, macrophage, microglia, lymphoid, and mast cells (Piomelli, 2003). Expression of endocannabinoid receptors on various CNS cell types is shown in Figure 1.

The most studied endogenous ligands that bind cannabinoid receptors are AEA (Devane *et al*, 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al*, 1995; Sugiura *et al*, 1995). Although several biosynthetic pathways have been proposed for AEA production, rate-limiting biosynthetic enzyme(s) remain to be identified (Blankman and Cravatt, 2013). In contrast, diacylglycerol lipase (DAGL) α and β transform diacylglycerols into 2-AG (Bisogno *et al*, 2003; Gao *et al*, 2010; Tanimura *et al*, 2010). AEA and 2-AG are rapidly hydrolyzed by fatty acid amide hydrolase (FAAH) (Cravatt *et al*, 1996, 2001), and monoacylglycerol lipase (MAGL) (Dinh *et al*, 2002), respectively. Besides serving as the major catabolic enzyme of AEA, FAAH hydrolyzes other bioactive lipids, such as *N*-palmitoylethanolamine (PEA) (Fezza *et al*, 2008), which does not bind cannabinoid receptors, but functions as an endogenous ligand for peroxisome proliferator receptor- α (PPAR- α) (Lo Verme *et al*, 2005). In addition to being the primary hydrolytic enzyme for 2-AG (Dinh *et al*, 2002), MAGL is a rate-limiting enzyme in the production of free arachidonic acid in brain, liver, and lung, but not in the gastrointestinal tract or other organs (Nomura *et al*, 2011). Thus, MAGL inhibitors can produce antinociceptive and anti-inflammatory actions through increased levels of 2-AG-stimulating cannabinoid receptors, and decreased levels of arachidonic acid and its pro-inflammatory metabolites. A schematic representation of key endocannabinoid-regulating enzymes is presented in Figure 2.

Here we review current knowledge regarding the antinociceptive effects of cannabinoids in inflammatory and neuropathic preclinical studies of pain as well as clinical evaluations. It is noteworthy that there is a growing interest

in potential therapeutic effects of another cannabis constituent, cannabidiol (CBD), which is included in Sativex, a drug containing approximately equal parts of THC and CBD. Preclinical studies testing combinations of THC and CBD, as well as clinical studies using Sativex to demonstrate anti-inflammatory (Lodzki *et al*, 2003; Malfait *et al*, 2000; Xiong *et al*, 2012) and antinociceptive effects (King *et al*, 2017; Langford *et al*, 2013; Lehmann *et al*, 2017; Serpell *et al*, 2014; Ward *et al*, 2014) are reported in the literature. However, as CBD does not bind cannabinoid receptors, this review will not discuss this phytocannabinoid at length. In particular, we focus on the well-established antinociceptive effects of cannabimimetic agents in carrageenan- and lipopolysaccharide (LPS)-induced acute inflammatory pain models, as well as in longer-term inflammatory pain models (for example, complete Freund's adjuvant-induced chronic inflammation and collagen-induced arthritis). We also discuss neuropathic pain associated with nerve injury, diabetes, chemotherapeutic agents, and migraine. Table 1 provides an overview of a selected range of rodent assays to assess nociceptive behavior in preclinical inflammatory and neuropathic pain models. Furthermore, we cover the opioid-sparing effects of cannabinoids in terms of cannabinoid effectiveness in reducing opioid doses, thereby reducing side effects of each drug without reducing overall antinociceptive efficacy. Finally, assorted clinical trials testing cannabis in neuropathic pain patients are discussed.

ACUTE AND CHRONIC INFLAMMATORY PAIN

Anti-Inflammatory Effects: *In Vitro* Assays

Pharmacological agents acting on various components of the endocannabinoid system exert anti-inflammatory effects, primarily through suppression of cytokine production, inhibition of cell proliferation, and induction of cell apoptosis (Nagarkatti *et al*, 2009). The inflammatory response to insult must be tightly regulated in order to minimize damage to healthy tissues. Thus, in addition to proinflammatory cytokines, activated immune cells produce and release anti-inflammatory mediators, including interleukin-10 (IL-10), which are regulated by the endocannabinoid system (Klein, 2005). Endocannabinoids mediate inflammation by regulating cytokines at different steps throughout the inflammatory response (Cabral and Griffin-Thomas, 2009). For example, THC and AEA suppress proinflammatory cytokines and enhance anti-inflammatory cytokines in both innate and adaptive immune responses (Cabral and Griffin-Thomas, 2009). AEA inhibits microglial nitric oxide (NO) synthesis through the mitogen-activated protein kinase pathway (Eljaschewitsch *et al*, 2006) and inhibits tumor necrosis factor α (TNF- α) activation of the transcription nuclear factor kappa B (Sancho *et al*, 2003). However, cannabinoids may also increase production of proinflammatory cytokines, depending on the model, dose, and drug probe (Klein, 2005). For example, *in vitro* studies THC inhibits proinflammatory

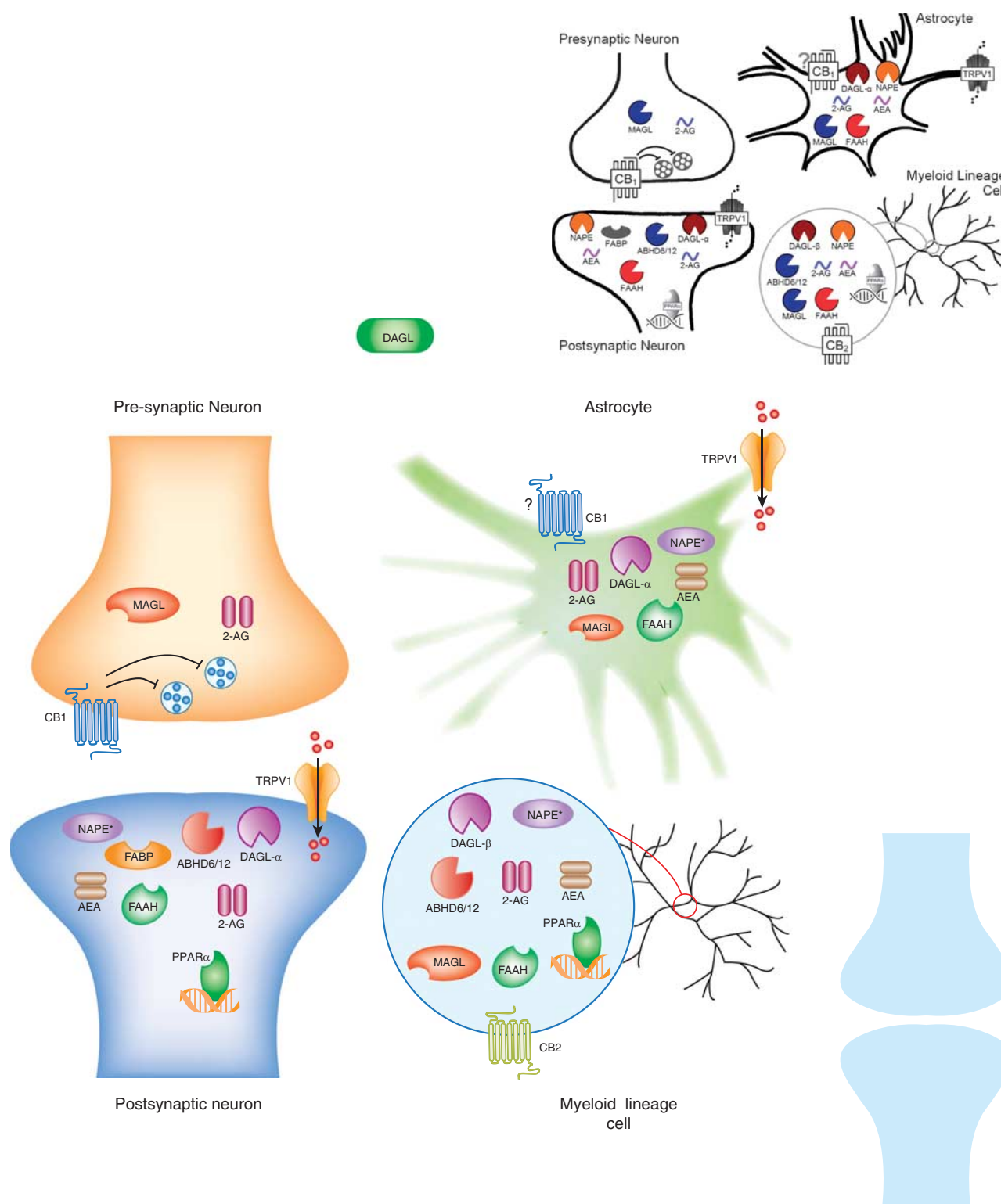


Figure 1. Endocannabinoid system localization by CNS cell type. Drugs acting upon cannabinoid receptors and the endocannabinoid-regulating enzymes are determined not only by drug class, efficacy, affinity, and potency, but also by cellular compartmentalization of the drug target. 2-AG, 2-arachidonyl glycerol; ABHD6, α/β -hydrolase domain-6; ABHD12, α/β -hydrolase domain-12; AEA, anandamide; CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; DAGL- α , diacylglycerol lipase- α ; DAGL- β , diacylglycerol lipase- β ; FABP, fatty acid binding protein; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE, N-arachidonoyl phosphatidylethanolamine; PPAR α , peroxisome proliferator-activated receptor alpha; TRPV1, transient receptor potential cation channel subfamily V member 1. Question marks refer to conflicting evidence to support the targets cellular localization.

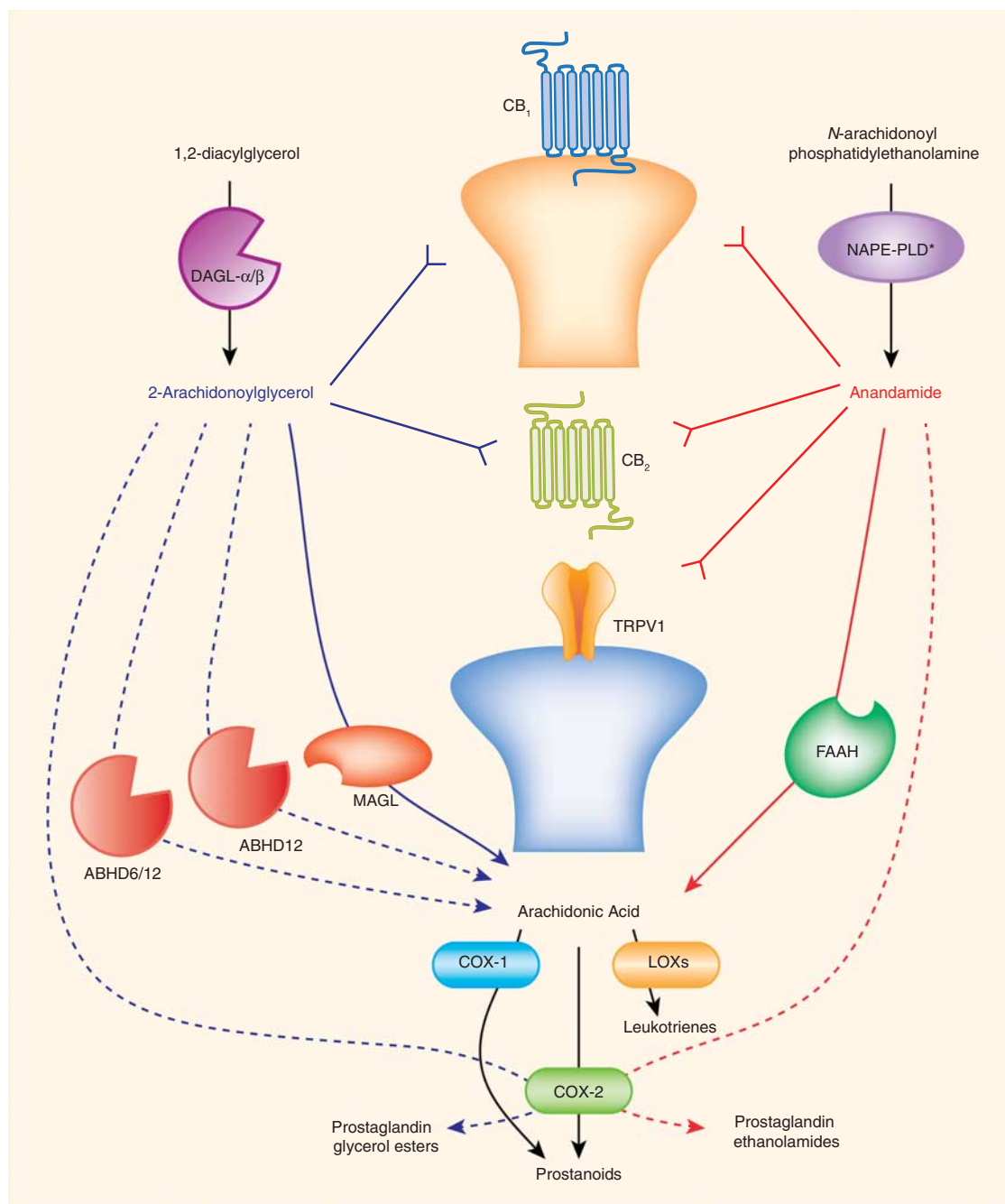


Figure 2. Enzymatic regulation of endocannabinoids and primary targets. Endogenous cannabinoids are enzymatically regulated, produced, and released on demand. Anandamide and 2-AG bind and activate CB₁ and CB₂ receptors. Anandamide also binds and activates transient receptor potential cation channel subfamily V member 1 (TRPV1). Diacylglycerol lipase (DAGL) alpha and beta synthesize 2-arachidonoylglycerol. *N*-acyl phosphatidylethanolamine-selective phospholipase D (NAPE-PLD*) is one of several enzymes proposed to synthesize anandamide. Monoacylglycerol lipase (MAGL), alpha/beta-hydrolase domain containing (ABHD) 6 and 12 hydrolyze 2-AG to create arachidonic acid. Several enzymes, including cyclooxygenase (COX) 1 and 2 and lipoxygenases (LOXs) convert arachidonic acid into bioactive lipids. COX2 also converts anandamide to prostaglandin ethanolamides, and 2-AG to prostaglandin glycerol esters. Thick blue or red lines with arrows represent major degradative pathways for 2-AG and anandamide, respectively. Dashed lines with arrows represent other degradative pathways. Reverse arrows (Y) represent activation of receptor targets of each endocannabinoid.

cytokine synthesis at nanomolar concentrations, but stimulates proinflammatory cytokine synthesis at micromolar concentrations, indicating a biphasic effect (Berdyshev *et al*, 1997).

Macrophages are the main producers of proinflammatory cytokines, especially during the early stages of the innate

immune response. THC and AEA attenuate LPS-stimulated IL-6, and NO release from macrophages *in vitro* (Chang *et al*, 2001). Similarly, during the adaptive immune response, T cells produce cytokines. The endocannabinoid system also modulates inflammation by shifting the balance of CD4⁺ 'Helper' T cells through suppression of Th1 proinflammatory

TABLE 1 Assays to Evaluate Nociceptive Behavior in Rodents

Test	Stimulus	Description	Ref
Tail flick	Thermal, heat	The measured parameter is the latency, in seconds, for tail flick reflex following tail exposure to a heat stimulus.	D'Amour and Smith, 1941
Hot plate	Thermal, heat	The measured parameter is usually the latency for paw licking or the first observed response (ie, jump) when the animal is placed on a hot surface (52 or 55 °C).	O'Callaghan and Holtzman, 1975
Acetone test	Thermal, cold	The measured parameter is usually the number or frequency of brisk foot withdrawal after applying a drop of acetone to a hind paw.	Choi <i>et al</i> , 1994
Von-Frey test	Mechanical	The measured parameter is usually the withdrawal threshold (g) after the randomly application of a series of calibrated monofilaments on the hind paws for 3 s.	Murphy <i>et al</i> , 1999
Randal-Selitto	Mechanical	The measured parameter is usually the withdrawal threshold (g) or vocalization when the hind paw is placed between a fixed element, such as a surface or a blunt point, and a mobile blunt point exerting a controlled pressure.	Randall and Selitto, 1957
Conditioned place preference (CPP) Conditioned place avoidance (CPA)	Motivational	During a preconditioning phase, subjects are placed in a box consisting of a neutral middle chamber and two chambers on either side (visually, texturally, and olfactory different). Drug conditioning trials are run with the drug given in one of the two chambers, which provides the opportunity for the development of an association. During testing, amount of time spent in the drug-paired chamber indicates a preference. The measured parameter is the place preference score (PPS).	Roux <i>et al</i> , 2003
Intracranial self-administration (ICSS)	Motivational	Under pain condition, animals are provided with intravenous catheters and allowed to perform an operant response such as the depression of a lever to self-administer a drug. This technique has been used extensively to evaluate a drug's propensity for abuse.	Carlezon and Chartoff, 2007
Nesting test	Pain-depressed behavior	The measured parameter is usually the number of area cleared in the animal cage wherein 6 nestlet pieces are placed in a period of 100 s. The nesting procedure is an evaluation of pain-related depressed behavior.	Negus <i>et al</i> , 2015

activity and promotion of Th2 anti-inflammatory activity (Yuan *et al*, 2002). THC decreases *Legionella pneumophila*-induced production of the proinflammatory cytokines, gamma interferon (IFN- γ) and IL-12, typically released from the Th1 cells, and increase production of the anti-inflammatory cytokine IL-4, released by Th2 cells in mouse splenocytes. Selective antagonists of both the CB₂ receptor, SR144528, and CB₁ receptor, rimonabant, reverses the suppression of IFN- γ and IL-12, indicating that these receptors play necessary roles in the modulation of proinflammatory cytokines (Klein *et al*, 1985). These results suggest that endogenous cannabinoids inhibit the production of proinflammatory cytokines and increase the production of anti-inflammatory cytokines throughout the inflammatory response.

The endocannabinoid system also modulates the inflammatory response through the suppression of immune cell activation, proliferation and migration, and the activation of immune cell apoptosis. Administration of the cannabinoid receptor agonist, CP55,940 decreased the migration of rat macrophages through a CB₁ and CB₂ receptor mechanism in both *in vivo* and *in vitro* models (Sacerdote *et al*, 2000). Furthermore, THC indirectly inhibits the activation of T helper cells by suppressing antigen presentation in macrophages (McCoy *et al*, 1995).

Cannabinoids also inhibit the cell-specific proliferation of B and T cells (Cabral and Griffin-Thomas, 2009; Klein and Cabral, 2006). For example, THC inhibited the proliferation of human T cells stimulated with antigen-primed dendritic cells (Yuan *et al*, 2002). Similarly, THC inhibits the proliferation of mouse splenic T cells stimulated

by concanavalin A, and B cells stimulated by LPS (Klein *et al*, 1985). In addition to inhibiting cell proliferation, THC induces apoptosis of mouse macrophages, T cells, and B cells in primary splenic and thymic cultures (McKallip *et al*, 2002).

Cannabinoids elicit analgesic and immunomodulatory effects in arthritis and other inflammatory diseases. Fibroblast-like synoviocytes (FLS) reside in synovial tissue. FLSs from patients affected by rheumatoid arthritis and osteoarthritis express the CB₁ and CB₂ receptors (Richardson *et al*, 2008). Both receptors are expressed on chondrocytes and within the joint cartilage and subchondral bone of patients with osteoarthritis (Dunn *et al*, 2014, 2016). Within rheumatoid arthritis synovial tissue, CB₂ receptors are expressed on macrophages, CD4⁺ T cells, CD8⁺ T cells, and B cells (Fukuda *et al*, 2014). Interestingly, AEA and 2-AG are present in the synovial fluid of patients affected by both rheumatoid arthritis and osteoarthritis, but not in healthy controls, suggesting an upregulation in response to inflammation and cartilage degradation (Richardson *et al*, 2008). The presence of cannabinoid receptors in arthritic joints suggests that the endocannabinoid system plays a compensatory role in this disease. However, the lack of highly selective CB₂ receptor antibodies limits interpretation of some studies. Thus, the use of stringent negative controls is crucial for the correct detection and quantification of protein targets (Marchalant *et al*, 2014).

In arthritic synovial tissue, FLSs release matrix metalloproteinases that degrade the cartilage extracellular matrix and pro-inflammatory cytokines (Karouzakis *et al*, 2006). The release of matrix metalloproteinases and cytokines are

attenuated by cannabinoids *in vitro* (Lowin *et al*, 2015, 2016; Selvi *et al*, 2008). In FLSs derived from patients with rheumatoid arthritis and osteoarthritis, synthetic cannabinoid receptor agonists (CP55,940, WIN55,212, or Ajulemic acid) or AEA (Lowin *et al*, 2015) attenuate TNF- α stimulated production of the proinflammatory cytokines IL-6 and IL-8, and matrix degrading enzymes matrix metalloproteinases-1, -3, and -9 (Johnson *et al*, 2007; Lowin *et al*, 2015, 2016; Selvi *et al*, 2008). The selective CB₂ receptor agonists HU-308 and JWH133 also reduce IL-6, IL-8, and matrix metalloproteinases-3 from TNF α or IL-1 β stimulated rheumatoid arthritis and osteoarthritis FLS (Fukuda *et al*, 2014; Gui *et al*, 2014). AEA's attenuation of IL-6 and IL-8 is blocked by the TRPA1 antagonist A967079, but not by CB₁ or CB₂ receptor antagonists (Lowin *et al*, 2015), indicating that cannabinoids modulate inflammation through multiple pathways.

In rheumatoid arthritis FLSs stimulated by TNF α , AEA attenuates the phosphorylation of p38 and ERK1/2, but not cJUN, indicating a decrease in mitogen-activated protein kinase activation (Lowin *et al*, 2015). The selective CB₂ receptor agonist HU-308 also inhibited IL-1 β stimulated phosphorylation of ERK 1/2 and p38 mitogen-activated protein kinase in FLS from rheumatoid arthritis patients (Gui *et al*, 2014). These studies suggest that cannabinoids may reduce arthritic inflammation and cartilage degradation by the release of proinflammatory cytokines and matrix-degrading enzymes from FLSs in the synovium through the modulation of ERK1/2 and p38 mitogen-activated protein kinase activity.

Cannabinoids may also reduce cartilage degradation in arthritis through the regulation of chondrocytes. In bovine nasal cartilage stimulated by IL-1 β , the cannabinoid receptor agonists WIN-55,212-2 and HU-210 prevented the breakdown of proteoglycan and collagen (Mbvundula *et al*, 2006). WIN-55,212-2 also inhibits the production of PGE₂, expression of iNOS, and the activation of nuclear factor kappa B in bovine chondrocytes (Mbvundula *et al*, 2006). Furthermore, incubation of WIN-55,212-2 alone or in combination with IL-1 β decreases the expression of matrix-degrading enzyme matrix metalloproteinases-3 and -13, and matrix metalloproteinases tissue inhibitor of metalloproteinase-1 and -2 in osteoarthritis chondrocytes (Dunn *et al*, 2014). WIN-55,212-2 also attenuates the production of the matrix-degrading enzyme disintegrin and metalloprotease with thrombospondin motifs-4 from osteoarthritis chondrocytes either unstimulated or stimulated with IL-1 β (Kong *et al*, 2016). The attenuation of the metalloprotease with thrombospondin motifs-4 production is reversed by the selective CB₂ receptor antagonist, JTE907, but not the selective CB₁ receptor antagonist, MJ15 indicating that CB₂ plays a necessary role in this effect. Taken together, these studies indicate that cannabinoids may reduce cartilage degradation by attenuating the release of matrix-degrading enzymes (that is, matrix metalloproteinases and metalloprotease with thrombospondin motifs) from chondrocytes.

Anti-Inflammatory Effects: *In Vivo* Assays

Acute pain models. Cannabinoids have anti-inflammatory properties and also elicit antinociceptive effects by inhibiting neuronal transmission in pain pathways. Cannabinoid-induced antinociception is reported in a variety of preclinical inflammatory pain models (Table 2; also see a review by Guindon and Hohmann, 2009), including intraplantar injection (that is, into the ventral footpad) of the seaweed extract carrageenan, and the Gram-negative bacterial-derived endotoxin lipopolysaccharide (LPS). These non-self inflammogens elicit an innate immune response that results in acute antinociception and localized edema in the affected paw. Several assays are available to test antinociceptive behavior in the animal models of inflammatory pain (Table 1). For example, the synthetic cannabinoid agonist HU-210 restores the carrageenan-induced reduction in weight bearing in rats (Clayton *et al*, 2002; Elmes *et al*, 2005; Sofia *et al*, 1973). Similarly, intraplantar injection of high-dose (that is, 25 μ g) LPS induces acute, localized pain and inflammation that is attenuated by synthetic cannabinoid agonists WIN55,212-2 and CP55,940 (Kinsey *et al*, 2011a; Naidu *et al*, 2010). These inflammatory pain assays, along with others, have been used to investigate the acute antinociceptive effects of cannabinoid receptor agonists, CB₁ receptor-positive allosteric modulators (PAMs), inhibitors of endocannabinoid-regulating enzymes, and inhibitors of endocannabinoid transport.

CB₂ receptor-selective agonists represent a promising strategy to bypass the undesirable cognitive and behavioral side effects of mixed CB₁/CB₂ receptor agonists. The selective CB₂ receptor agonists GW405833 and JWH133 attenuate edema and restore weight bearing in rat paws injected with carrageenan (Clayton *et al*, 2002; Elmes *et al*, 2005). The CB₂ receptor agonist O-3223 attenuates hyperalgesia and edema induced by intraplantar injection of LPS, and was reversed by the CB₂ receptor antagonist, SR144528, but not by rimobant (Kinsey *et al*, 2011a).

CB₁ receptor PAMs represent another approach to block inflammatory pain without cannabimimetic side effects. For example, the CB₁ positive allosteric modulator ZCZ011 increases binding of orthosteric CB₁ receptor agonists (Ignatowska-Jankowska *et al*, 2015b). When administered alone, ZCZ011 attenuates carrageenan-induced mechanical allodynia, but does not elicit anti-edematous effects, mediated by CB₂ receptors. The observations that ZCZ011 does not elicit psychomimetic effects in tetrad assay or substitute for cannabinoids in the drug discrimination paradigm, suggesting that this compound has a reduced side effect profile compared with orthosteric CB₁ receptor agonists (Ignatowska-Jankowska *et al*, 2015b).

Inhibiting endocannabinoid catabolic enzymes represents a distinct approach from direct-acting cannabinoid receptor agonists. In general, inhibition of FAAH or MAGL attenuates acute inflammatory pain with reduced cannabimimetic side effects compared with direct-acting CB₁ receptor agonists. For example, pharmacological inhibition

TABLE 2 Antinociceptive Effects of Cannabinoids in Pre-Clinical Models of Acute Inflammatory Pain

Pain model	Type	Treatment	Route	Species	Mechanical	Thermal	Weight bearing	Mechanism of action	Edema	Edema receptor	Citation
Carrageenan	Agonist	THC	p.o.	Rat	N/A	N/A	N/A	N/A	Decrease	N/A	Sofia et al, 1973
		HU210	i.p.	Rat	N/A	N/A	yes	CB ₁	Decrease	CB ₁ & CB ₂	Clayton et al, 2002; Elmes et al, 2005
	Phytocannabinoid	THCV	i.p.	Mouse	N/A	Plantar	N/A	Non-CB	Decrease	CB ₂	Bolognini et al, 2010
	CB ₁ agonist	ACEA	i.p.l.	Rat	von Frey	Plantar	N/A	CB ₁	N/A	N/A	Gutierrez et al, 2007
	CB ₁ PAM	ZCZ011	i.p.	Mouse	von Frey	N/A	N/A	CB ₁	No Δ	N/A	Ignatowska-Jankowska et al, 2015c
	CB ₂ agonist	GW405833	i.p.	Rat	N/A	N/A	yes	CB ₂	Decrease	CB ₂	Clayton et al, 2002
		JWH133	i.p.	Rat	N/A	N/A	yes	CB ₂	Decrease	CB ₂	Elmes et al, 2005
		AM1241	i.p.	Rat	N/A	Plantar	N/A	CB ₂	No Δ	N/A	Bingham et al, 2007; Nackley et al, 2003; Quartilho et al, 2003
		AM1241	i.p.l.	Rat	von Frey	Plantar	N/A	CB ₂	N/A	N/A	Quartilho et al, 2003 (plantar only); Gutierrez et al, 2007; Nackley et al, 2003 (von Frey and plantar)
	CB ₂ inverse agonist	JTE-907		Mouse	N/A	N/A	N/A	N/A	Decrease	N/A	Iwamura et al, 2001
	CB ₂ agonist + CB ₁ agonist	AM1241 + ACEA	i.p.l.	Rat	N/A	Plantar	N/A	N/A	Decrease	CB ₂	Gutierrez et al, 2007
	FAAH inhibition	FAAH KO	N/A	Mouse	N/A	Plantar	N/A	CB ₂	Decrease	CB ₂	Lichtman et al, 2004; Wise et al, 2008
		URB597	i.p.	Mouse	N/A	No	N/A	N/A	Decrease	CB ₂	Holt et al, 2005 (edema only); Costa et al, 2010
		URB597	i.p.l.	Rat	N/A	N/A	yes	PPAR-α	No Δ	N/A	Jhaveri et al, 2008
		URB937	i.p.	Mouse	von Frey & pressure	Plantar	N/A	CB ₁	Decrease	CB ₁ & CB ₂	Clapper et al, 2010
		JNJ-1661010		Rat	N/A	Plantar	N/A	N/A	N/A	N/A	Karbarz et al, 2009
		PF-3845	i.p.	Mouse	von Frey	N/A	N/A	N/A	Decrease	N/A	Ghosh et al, 2013
	MAGL inhibition	URB602	i.p.	Mouse	N/A	Plantar	N/A	CB ₂	Decrease	CB ₂	Comelli et al, 2007
		JZL184	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	Decrease	CB ₂	Ghosh et al, 2013
		KML129	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	Decrease	CB ₂	Ignatowska-Jankowska et al, 2014
		SBFI26	i.p.	Mouse	N/A	Plantar	N/A	N/A	Decrease	N/A	Kaczocha et al, 2014
	FABP	SBFI50	i.p.	Mouse	N/A	Plantar	N/A	N/A	Decrease	N/A	Kaczocha et al, 2014
		FABP5/7 KO	N/A	Mouse	N/A	Plantar	N/A	PPAR-α & TRPV1	Decrease	N/A	Kaczocha et al, 2015
	FAAH inhibition + NSAID	URB937 + Indomethacin	p.o.	Mouse	Pressure	Plantar	N/A	N/A	Decrease	N/A	Sasso et al, 2012
		PF-3845 + Diclofenac	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	N/A	N/A	Grim et al, 2014
	FAAH & MAGL inhibition	PF-3845 + JZL184	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	Decrease	CB ₂	Ghosh et al, 2015
		SA-57	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	Decrease	CB ₂	Wilkerson et al, 2017
	FAAH inhibition + TRPV1 antagonism	AA-5-HT	i.p.	Mouse	Randall-Stiletto	Plantar	N/A	CB ₁ & TRPV1	Decrease	TRPV1	Costa et al, 2010
	FAAH inhibition + TRPV1 antagonism	AA-5-HT	i.p.l.	Mouse	no	No	N/A	N/A	N/A	N/A	Costa et al, 2010
	FAAH & sHE inhibition	URB937 + TPPU	p.o.	Mouse	pressure	Plantar	N/A	N/A	Decrease	N/A	Sasso et al, 2015
LPS (high dose)	Agonist	WIN55212-2	i.p.	Mouse	N/A	HP	N/A	N/A	Decrease	N/A	Naidu et al, 2010
		CP55,940	i.p.	Mouse	N/A	HP	N/A	CB ₁ & CB ₂	Decrease	CB ₂	Kinsey et al, 2011a
	CB ₂ agonist	O-3223	i.p.	Mouse	N/A	HP	N/A	CB ₂	Decrease	CB ₂	Kinsey et al, 2011a

Table 2 (Continued)

Pain model	Type	Treatment	Route	Species	Mechanical	Thermal	Weight bearing	Mechanism of action	Edema	Edema receptor	Citation
LPS (high dose)	FAAH inhibition	FAAH KO URB597	N/A i.p.	Mouse Mouse	N/A N/A	HP HP	N/A N/A	CB ₁ CB ₁	Decrease Decrease	CB ₂ CB ₂	Naidu <i>et al</i> , 2010 Naidu <i>et al</i> , 2010
LPS (low dose)	FAAH inhibition	FAAH KO URB597	N/A i.p.	Mouse Mouse	von Frey von Frey	N/A N/A	N/A N/A	CB ₁ & CB ₂ N/A	N/A N/A	N/A N/A	Booker <i>et al</i> , 2012 Booker <i>et al</i> , 2012
		OL-135	i.p.	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Booker <i>et al</i> , 2012
		PF-3845	i.p.	Mouse	von Frey	N/A	N/A	CB ₁ & CB ₂	N/A	N/A	Booker <i>et al</i> , 2012
		PF-3845	i.p.l.	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Booker <i>et al</i> , 2012
	DAGL-β inhibition	DAGL-β KO	N/A	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Wilkerson <i>et al</i> , 2016a
		KT-109	i.p.	Mouse	von Frey	N/A	N/A	Non-CB	N/A	N/A	Wilkerson <i>et al</i> , 2016a
		KT-109	i.p.l.	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Wilkerson <i>et al</i> , 2016a
	ABHD6 inhibition	KT-195	i.p.	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Wilkerson <i>et al</i> , 2016a
	Agonist	THC	i.p.	Mouse	von Frey	N/A	N/A	N/A	N/A	N/A	Booker <i>et al</i> , 2012

Abbreviations: HP, hot plate; i.p., intraperitoneal; i.p.l., intraplantar; N/A, not assessed; non-CB, not blocked by CB₁ or CB₂ receptors; p.o., given via gavage.

or genetic deletion of FAAH attenuates carrageenan-induced inflammation as well as mechanical allodynia and thermal hyperalgesia (increased sensitivity to a noxious heat stimuli) (Ghosh *et al*, 2013; Karbarz *et al*, 2009; Lichtman *et al*, 2004). FAAH inhibitors also attenuate carrageenan-induced edema, an effect that is mediated through the CB₂ receptor (Holt *et al*, 2005; Lichtman *et al*, 2004). The FAAH inhibitor URB597, or genetic deletion of FAAH, also attenuates LPS-induced hyperalgesia and edema (Naidu *et al*, 2010). The anti-hyperalgesic effect of FAAH inhibition in the LPS model was reversed by CB₁ receptor antagonism, whereas the anti-edematous effect was reversed by CB₂ antagonism (Naidu *et al*, 2010). Similarly, the peripherally restricted FAAH inhibitor URB937 attenuates carrageenan-induced allodynia and hyperalgesia through a CB₁ mechanism of action. However, unlike brain-permeating FAAH inhibitors, the anti-edematous effects of URB937 are mediated by both CB₁ and CB₂ receptors (Clapper *et al*, 2010).

Similarly, inhibition of MAGL, the primary catabolic enzyme of 2-AG (Blankman *et al*, 2007), attenuates carrageenan-induced, acute paw edema and inflammatory pain (Comelli *et al*, 2007; Ghosh *et al*, 2013; Ignatowska-Jankowska *et al*, 2014). The selective MAGL inhibitors JZL184 and KML129 attenuate carrageenan-induced mechanical allodynia through CB₁ and CB₂ receptor-dependent mechanisms, but only CB₂ receptors mediate the anti-edematous effects of these inhibitors (Ghosh *et al*, 2013; Ignatowska-Jankowska *et al*, 2014). Combining a high dose of the FAAH inhibitor, PF-3845 with a low dose of the MAGL inhibitor, JZL184, attenuates carrageenan induced mechanical allodynia and paw edema in mice to a greater degree than either drug alone (Ghosh *et al*, 2015). Similarly, the dual FAAH/MAGL inhibitor SA-57 attenuates allodynia and edema induced by intraplantar carrageenan (Wilkerson *et al*, 2017). The anti-allodynic effects of combined FAAH/MAGL inhibition are reversed by either CB₁ or CB₂ receptor antagonists, whereas only a CB₂ receptor antagonist reverses the anti-edematous effects of dual FAAH/MAGL inhibition.

FAAH inhibition has also been combined with other, non-cannabinoid analgesics to attenuate edema and pain in the carrageenan model. For example, AA-5-HT, a dual FAAH inhibitor/transient receptor potential cation channel sub-family V member 1 (TRPV1) antagonist, attenuates thermal and mechanical hyperalgesia, as well as edema produced by an intraplantar injection of carrageenan (Costa *et al*, 2010). Epoxidized fatty acids exert anti-inflammatory and antinociceptive effects, and are metabolized by the enzyme soluble epoxide hydrolase. Combined administration of the peripherally-restricted FAAH inhibitor, URB937, and the epoxide hydrolase inhibitor, TPPU, attenuates carrageenan-induced mechanical and thermal hyperalgesia, as well as, paw edema, in a synergistic manner (Sasso *et al*, 2015). Similarly, dual administration of URB937 and the nonsteroidal anti-inflammatory drug (NSAID) indomethacin synergistically attenuates hyperalgesia, allodynia, and edema

induced by intraplantar carrageenan injection (Sasso *et al*, 2012). Mechanical allodynia induced by carrageenan injection is also reduced by coadministration of the FAAH inhibitor PF-3845 and the NSAID diclofenac (Grim *et al*, 2014). The anti-allodynic effect is blocked by SR144528 or rimonabant, indicating that both cannabinoid receptors mediate this antinociceptive effect (Grim *et al*, 2014).

Fatty acid-binding proteins (FABPs) are proposed to transport endocannabinoids intracellularly, from the cell membrane to the endoplasmic reticulum for hydrolysis (Kaczocha *et al*, 2009). The FABP inhibitors SBF126 and SBF150 attenuate carrageenan-induced acute paw edema and thermal hyperalgesia (Kaczocha *et al*, 2014). Similarly, genetic deletion of FABP5 and FABP7 reduces thermal hyperalgesia and edema induced by carrageenan injection (Kaczocha *et al*, 2015). PPAR- α and TRPV1 receptors mediate the anti-hyperalgesic phenotypes of FABP5 ($-/-$) mice and FABP7 ($-/-$) mice. These data suggest that preventing endocannabinoid degradation through inhibition of either their catabolic enzymes or FABPs attenuates acute inflammatory pain.

Localized administration of low dose (ie, 2.5 μ g) LPS evokes tactile allodynia that is attenuated by FAAH inhibition without inducing paw edema (Booker *et al*, 2012). Genetic deletion of FAAH also reduces mechanical allodynia induced by low-dose LPS, but this anti-allodynia is absent in mice expressing FAAH only in nervous tissue (Booker *et al*, 2012) and is mediated by both CB₁ and CB₂ receptors. Intraplantar administration of PF-3845 also attenuates LPS-induced allodynia (Booker *et al*, 2012). However, inhibition of ABHD6, an enzyme that plays a minor role in 2-AG degradation (Blankman *et al*, 2007), does not affect LPS-induced allodynia (Wilkerson *et al*, 2016a).

Blockade of the 2-AG biosynthetic enzyme DAGL- β also produces antinociception in the LPS model of inflammatory pain. Systemic or localized administration of the DAGL- β inhibitor KT-109 reversed mechanical allodynia induced by intraplantar injection of low-dose LPS (Wilkerson *et al*, 2016a). Moreover, DAGL- β ($-/-$) mice displayed an antinociceptive phenotype in this model. The antinociceptive effects following pharmacological inhibition or genetic deletion of DAGL- β are possibly the result of reduced production of proinflammatory metabolites of arachidonic acid in macrophages. Specifically, DAGL- β is highly expressed in macrophages, and its blockade leads to reduced levels of 2-AG, arachidonic acid, and a variety of proinflammatory arachidonic acid metabolites and proinflammatory cytokines in these cells (Hsu *et al*, 2012). Importantly, DAGL- β inhibition also blocks the LPS-induced production of proinflammatory mediators (Hsu *et al*, 2012). Thus, increasing 2-AG levels via MAGL inhibition reduces LPS-induced nociception through a cannabinoid receptor mechanism of action, while decreasing 2-AG levels in peripheral tissue (possibly in macrophages) by inhibiting DAGL- β reduces LPS-induced nociception by dampening the innate immune response.

Chronic pain models. Unlike carrageenan or LPS that only induce inflammatory pain for a period of hours to a few days, other inflammatory pain models elicit nociceptive behavior that may persist for weeks. Several well-characterized long-term inflammatory pain models include the complete Freund's adjuvant (CFA) model, which induces nociception and paw swelling, osteoarthritis, produced by intra-articular injection of monosodium iodoacetate (MIA), and the collagen-induced arthritis (CIA) model, a well-characterized mouse model of inflammatory arthritis. These models of chronic inflammatory pain lead to a variety of changes in the endocannabinoid system. Moreover, a wide range of cannabimimetic agents produce antinociception in CFA and CIA inflammatory pain models (Table 3), as well as osteoarthritis models (Table 4).

CFA administration into a ventral footpad leads to upregulation of CB₂ receptors, but not CB₂ receptor mRNA in dorsal root ganglia or ipsilateral paw tissue of rats, suggesting that these receptors play an integral role in the endocannabinoid modulation of chronic inflammatory pain (Hsieh *et al*, 2011). Acute administration of CB₂ receptor agonists reduces CFA-induced mechanical and thermal hyperalgesia (Hsieh *et al*, 2011; Valenzano *et al*, 2005; Yao *et al*, 2008, 2009). Furthermore, intraplantar administration of the CB₂ receptor agonist JWH015 reduces allodynia and hyperalgesia induced by CFA injection (Negrete *et al*, 2011). Unexpectedly, the antinociceptive effects of this drug are mediated by CB₂ receptors and μ opioid receptors (MORs). The observation that CFA leads to increased expression of these receptors in the paw and dorsal root ganglia may account for the recruitment of MORs (Negrete *et al*, 2011). Systemic or intrathecal administration of NMP-181, which acts as a CB₂ receptor agonist and T-type channel inhibitor, also attenuates CFA-induced mechanical hyperalgesia (Gadotti *et al*, 2013). In another study, the CB₂ receptor agonist GW405833 was reported to produce antinociceptive effects through CB₁ receptor mechanism of action, while CB₂ receptors were dispensable (Li *et al*, 2017). Selective CB₂ receptor agonists often possess low CB₁ receptor affinity, and when administered *in vivo* may activate both receptors, particularly when administered at high doses. Thus, studies employing CB₂ receptor-selective agonists need to apply caution by testing whether CB₁ receptors contribute to any observed effects.

Although CFA leads to an upregulation of CB₂ receptors, CB₁ receptor stimulation also attenuates chronic inflammatory pain. Localized administration of the CB₁ receptor agonist ACPA attenuates CFA-induced mechanical hyperalgesia (Auh *et al*, 2016). Combination of ACPA and the MOR agonist DAMGO reduces CFA-induced hyperalgesia. However, isobolographic analyses revealed that the interaction is infra-additive, indicating that the combination is less effective than either drug by itself (Auh *et al*, 2016).

In agreement with studies employing acute inflammatory pain models, inhibition of endocannabinoid catabolic enzymes also reduces chronic inflammatory pain. For example, FAAH inhibition attenuates mechanically-induced

TABLE 3 Antinociceptive Effects of Cannabinoids in Pre-Clinical Models of Chronic Inflammatory Pain

Pain model	Type	Treatment	Route	Species	Mechanical	Thermal	Pain receptor	Inflammation	Mechanism of action	Joint damage	Joint receptor	References
CIA	CBD derivatives	CBD	i.p. & p.o.	Mouse	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Malfait et al, 2000
		HU320	i.p.	Mouse	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Sumariwalla et al, 2004
		HU-444	i.p. & p.o.	Mouse	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Haj et al, 2015
	CB ₂ agonist	JWH133	i.p.	Mouse	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Fukuda et al, 2014
		HU-308	i.p.	Mouse	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Gui et al, 2015
	FAAH inhibition	FAAH KO	N/A	Mouse	N/A	HP & Tail immersion	N/A	Decrease	CB ₂	Decrease	CB ₂	Kinsey et al, 2011b
		URB597	i.p.	Mouse	N/A	HP & Tail immersion	CB ₁	Decrease	N/A	Decrease	N/A	Kinsey et al, 2011b
AIA	Agonist	JNJ1661010	i.p.	Mouse	N/A	N/A	N/A	Decrease	N/A	N/A	N/A	Lowin et al, 2015
		THC	i.p. & p.o. (swelling)	Rat	Pressure	N/A	CB ₁ , CB ₂ , & opioid	Decrease	N/A	N/A	N/A	Sofia et al, 1973 (swelling); Cox et al, 2007; Cox and Welch, 2004; Smith et al, 1998
		Ajulemic acid	p.o.	Rat	N/A	N/A	N/A	Decrease	N/A	Decrease	N/A	Zurier et al, 1998
CFA	Agonist	AEA	i.p.	Rat	Pressure	N/A	Non-CB ₁	N/A	N/A	N/A	N/A	Smith et al, 1998
		WIN55212-2	s.c.	Mouse	von Frey	Plantar	CB ₁ & CB ₂	N/A	N/A	N/A	N/A	Anderson et al, 2014
		HU-210	i.p.	Rat	von Frey	Plantar	N/A	N/A	N/A	N/A	N/A	Jayamanne et al, 2006
	CB ₁ agonist	ACPA	i.pl.	Rat	Randall-Selitto	N/A	N/A	N/A	N/A	N/A	N/A	Auh et al, 2016
		A-796260	i.p.	Rat	N/A	Plantar	CB ₂	N/A	N/A	N/A	N/A	Yao et al, 2008
	CB ₂ agonist	GW405833	i.p.	Mouse & Rat	von Frey (M) & pressure (R)	N/A	CB ₂	N/A	CB ₁	N/A	N/A	Li et al, 2017; Valenzano et al, 2005; Whiteside et al, 2005
		A-836339	i.p.	Rat	N/A	Plantar	CB ₂	N/A	N/A	N/A	N/A	Hsieh et al, 2011
		JWH015	i.pl.	Mouse	von Frey	Plantar	CB ₂ & MOR	N/A	N/A	N/A	N/A	Negrete et al, 2011
		AM1241	i.p.	Rat	N/A	Plantar	N/A	N/A	N/A	N/A	N/A	Hsieh et al, 2011
		URB597	i.p.	Rat	von Frey	Plantar	CB ₁ & CB ₂	N/A	N/A	N/A	N/A	Jayamanne et al, 2006
	FAAH inhibition	PF-3845	p.o.	Rat	von Frey	N/A	CB ₁ & CB ₂	N/A	N/A	N/A	N/A	Ahn et al, 2009
		PF-04457845	p.o.	Rat	von Frey	N/A	CB ₁ & CB ₂	N/A	N/A	N/A	N/A	Ahn et al, 2011
		URB-937	p.o.	Mouse	Pressure	Plantar	N/A	N/A	N/A	N/A	N/A	Sasso et al, 2012
	Multiple targets	AM404	s.c.	Rat	Randall-Selitto	Plantar	CB ₁	N/A	N/A	N/A	N/A	La Rana et al, 2008
		ACPA + DAMGO	i.pl.	Rat	Randall-Selitto; attenuate by antagonistic	N/A	N/A	N/A	N/A	N/A	N/A	Auh et al, 2016
	CB ₂ agonist + T-type channel inhibition	NMP-181	i.p. & i.t.	Mouse	DPA	N/A	N/A	N/A	N/A	N/A	N/A	Gadotti et al, 2013
		NMP-181	i.p. & i.t.	Mouse	DPA	N/A	N/A	N/A	N/A	N/A	N/A	Gadotti et al, 2013
	FAAH & MAGL inhibition	JZL195	s.c.	Mouse	von Frey	Plantar	CB ₁ & CB ₂	N/A	N/A	N/A	N/A	Anderson et al, 2014

Abbreviations: AIA, adjuvant-induced arthritis; HP, hot plate; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; p.o., given via gavage; MOR, μ -opioid receptor; N/A, not assessed; s.c., subcutaneous.

TABLE 4 Antinociceptive Effects of Cannabinoids in Preclinical Models of Osteoarthritis

Pain model	Type	Treatment	Route	Species	Mechanical	Mechanism of action	Weight bearing	WB receptor	Grip force	Joint damage	References
MIA	CB ₂ agonist	A-796260	i.p.	Rat	N/A	N/A	N/A	N/A	Yes	N/A	Yao <i>et al</i> , 2008
		JWH133	s.c.	Rat	von Frey	N/A	Yes	N/A	N/A	N/A	Burston <i>et al</i> , 2013
	CB ₂ antagonist	CB ₂ KO	N/A	Mouse	von Frey, augmented	N/A	N/A	N/A	N/A	No Δ	La Porta <i>et al</i> , 2013
	CB ₂ over expression	CB ₂ xP	N/A	Mouse	von Frey	N/A	N/A	N/A	N/A	no Δ	La Porta <i>et al</i> , 2013
	CB ₁ antagonist	CB ₁ KO	N/A	Mouse	von Frey, no Δ	N/A	N/A	N/A	N/A	No Δ	La Porta <i>et al</i> , 2013
	FAAH inhibition	URB597	s.c.	Rat	N/A	N/A	Yes	CB ₁	N/A	N/A	Schueler <i>et al</i> , 2011
		URB597	i.p.	Rat	PAM	N/A	N/A	N/A	N/A	N/A	Malek <i>et al</i> , 2016
		PF-04457845	p.o.	Rat	Randall-Selitto	N/A	N/A	N/A	N/A	N/A	Ahn <i>et al</i> , 2011
	MAGL inhibition	MJN110	i.p.	Rat	von Frey	CB ₁ & CB ₂	Yes	CB ₂	N/A	No Δ	Burston <i>et al</i> , 2016
	FAAH inhibition + TRPV1 antagonist	OMDM-198	i.p.	Rat	PAM	CB ₁ & TRPV1	N/A	N/A	N/A	N/A	Malek <i>et al</i> , 2016
DMM	CB ₂ agonist	HU-308	N/A	Mouse	N/A	N/A	N/A	N/A	N/A	Decrease	Sophocleous <i>et al</i> , 2015
	CB ₂ antagonist	CB ₂ KO	N/A	Mouse	N/A	N/A	N/A	N/A	N/A	Increase	Sophocleous <i>et al</i> , 2015
Spontaneous age related osteoarthritis	CB ₂ antagonist	CB ₂ KO	N/A	Mouse	N/A	N/A	N/A	N/A	N/A	Increase	Sophocleous <i>et al</i> , 2015

Abbreviations: xP, overexpression; i.p., intraperitoneal; s.c., subcutaneous; p.o., via gavage; no Δ , no change; N/A, not assessed.

pain in the CFA model of inflammatory pain (Ahn *et al*, 2009, 2011; Jayamanne *et al*, 2006; Sasso *et al*, 2012). These anti-allodynic effects are mediated by both the CB₁ and CB₂ receptors. The dual FAAH-MAGL inhibitor JZL195 reduces mechanical allodynia and thermal hyperalgesia induced by CFA injection (Anderson *et al*, 2014). The CB₁ receptor antagonist AM251 fully reverses these antinociceptive effects, while the CB₂ receptor antagonist AM630 partially reverses these effects (Anderson *et al*, 2014).

Administration of CBD, or its synthetic analogs, attenuates clinical signs of arthritis and joint damage, while having an immunosuppressant effect in CIA (Haj *et al*, 2015; Malfait *et al*, 2000; Sumariwalla *et al*, 2004). The selective CB₂ receptor agonists JWH133 and HU-308 attenuated paw swelling, cartilage degradation, and bone erosion in mice subjected to CIA (Fukuda *et al*, 2014; Gui *et al*, 2015). The anti-inflammatory and analgesic effects of FAAH inhibition have also been investigated in the CIA model. Wild-type mice treated with URB597 or FAAH (–/–) mice subjected to CIA show reduced thermal hyperalgesia in the hot plate and tail immersion tests. Furthermore, chronic FAAH inhibition reduces CIA-induced paw swelling (Kinsey *et al*, 2011b; Lowin *et al*, 2015) and joint destruction (Kinsey *et al*, 2011b).

Osteoarthritis is a highly prevalent type of arthritis characterized by synovitis, and degeneration of both articular cartilage and subchondral bone in the joints of the hands, knees, hips, and spine (Goldring and Goldring, 2007). MIA induces chondrocyte death, cartilage degradation, and chronic nociception (Burston *et al*, 2013). This nociception and joint damage occur ipsilateral, but not contralateral, to the injection site. Administration of MIA into the knee joint leads to upregulation of CB₂ and CB₁ receptor mRNA in the ipsilateral spinal cord of rats, suggesting that these receptors undergo compensatory changes in this osteoarthritis model, and may represent potential targets for novel osteoarthritis treatments (Burston *et al*, 2013; Malek *et al*, 2015).

After MIA administration, CB₂ (–/–) mice show augmented mechanical allodynia compared with wild-type littermates (La Porta *et al*, 2013). Interestingly, CB₂ (–/–) mice also display allodynia in the contralateral paw after MIA. This bilateral augmented allodynic response has also been reported to occur in CB₂ (–/–) mice subjected to sciatic nerve injury, indicating the mirrored response is not MIA model specific (Racz *et al*, 2008). By comparison, CB₁ (–/–) mice do not differ in allodynic response on the ipsilateral or contralateral paw after MIA, supporting the role of CB₂ in the development of allodynia in osteoarthritis (La Porta *et al*, 2013).

Mice overexpressing the CB₂ receptor (CB₂ xP) display an attenuated MIA-induced mechanical allodynic phenotype (La Porta *et al*, 2013). Furthermore, the CB₂ receptor agonist JWH133 attenuates MIA-induced allodynia and restores weight bearing on the arthritic joint (Burston *et al*, 2013). Intrathecal injection of JWH133 also attenuates the firing of wide dynamic range neurons after MIA injection, indicating CB₂ receptor activation in the spinal cord may attenuate MIA-induced allodynia (Burston *et al*, 2013).

Osteoarthritis can also be modeled in mice by destabilization of the medial meniscus by surgically sectioning a ligament in the joint. The CB₂ receptor agonist HU308 reduced joint damage in the DMM osteoarthritis model, whereas CB₂ (–/–) mice developed more joint damage than wild-type mice (Sophocleous *et al*, 2015). By contrast, CB₂ (–/–) mice, CB₂ xP mice, and CB₁ (–/–) mice subjected to MIA-induced arthritis do not differ from wild-type mice in the amount of joint damage (La Porta *et al*, 2013). These conflicting results may be attributed to variations in the progression of joint destruction in the different models.

Inhibition of the major endocannabinoid catabolic enzymes also attenuates pain in a rodent model of osteoarthritis. The FAAH inhibitor URB597 reduces MIA-induced mechanical hyperalgesia and restores weight bearing on the arthritic limb (Malek *et al*, 2015; Schuelert *et al*, 2011). OMDM-198, a combined FAAH inhibitor-TRPV1 antagonist, attenuates mechanical hyperalgesia elicited by MIA injection (Malek *et al*, 2015). Furthermore, MIA administration leads to upregulation of FAAH and TRPV1 mRNA in the ipsilateral spinal cord (Malek *et al*, 2015). The MAGL inhibitor MJN110 also attenuates mechanical allodynia and restores MIA limb weight bearing. The anti-allodynic effects of MJN110 were mediated by a CB₂ receptor mechanism of action, while CB₁ receptors did not play a necessary role in this effect. However, weight bearing was only partially blocked by the CB₂ receptor antagonist, SR144528, but fully blocked by rimonabant, indicating a difference in receptor mechanism (Burstion *et al*, 2016).

Two noteworthy caveats to preclinical inflammatory pain models are that anti-inflammatory treatments, including manipulation of the endocannabinoid system, are often as follows: (1) administered prior to the induction of inflammation and thus block development of inflammation; and (2) administered repeatedly for a few days (ie, subchronically). In addition, acute manipulation of the endocannabinoid system typically reduces pain measures via CB₁ receptor. These experimental limitations must be considered when translating results from preclinical research to the clinic.

NEUROPATHIC PAIN

Neuropathic pain is a severe chronic, debilitating condition associated with nerve injury (for example, structural, nutritional, toxic, infectious, or autoimmune damage) that also develops following lesions to the CNS or PNS insult. Neuropathic pain often manifests as a spontaneous burning, tingling, or shooting sensation, which can be amplified by noxious (pressure, heat, and cold), and otherwise innocuous (touch and warm or cool temperatures) mechanical and thermal stimuli (Jensen and Finnerup, 2014). Neuropathic pain pathophysiology is complex and includes functional alterations of the CNS and PNS (Cohen and Mao, 2014) in addition to neuro-immune interactions (Austin and Moalem-Taylor, 2010).

The dorsal horn of the spinal cord receives noxious sensory information from primary afferent Aδ fibers (ie, medium diameter myelinated afferents that transmit acute, localized sharp pain sensation) and C fibers (that is, small diameter unmyelinated afferents that convey poorly localized delayed pain sensation) (Braz *et al*, 2014). The largest-diameter, myelinated primary afferent Aβ fibers transmit innocuous mechanical stimuli (for example, light touch) and have their terminals in the deeper lamina of the dorsal horn and mainly target excitatory and inhibitory interneurons. On the other hand, the superficial lamina of the dorsal horn is abundant with terminals from C and Aδ fibers that activate projection neurons and excitatory interneurons. Under normal conditions, Aβ fibers do not activate nociceptive projection neurons and do not transmit pain because of strong suppression of signaling by inhibitory interneurons. However, under pathological conditions (that is, peripheral inflammation, or peripheral nerve injury; PNI), neuropathic pain may develop. Nerve injury induces hyperexcitability, thereby causing functional alterations in the neuronal network such as loss of function of inhibitory neurons in the dorsal horn, and results in neuropathic pain (Prescott *et al*, 2014; Todd, 2010). Additionally, glial cells (innate immune cells of the CNS), which include microglia and astrocytes, contribute to neuropathic pain by altering inflammation and glutamate signaling (Scholz and Woolf, 2007).

Despite recent progress, current mechanistic understanding of pain hypersensitivity caused by nerve damage remains limited. Traditional pain medications generally lack efficacy to treat neuropathic pain (Guirguis-Blake and Kelly, 2007), so a great need exists for the development of new efficacious analgesics. Also, laboratory animal models that accurately mimic key aspects of the pain reported clinically are needed to test candidate therapies. Assessing neuropathic pain behavior in rodents is crucial to validate pain models and new analgesics. Although a wide variety of laboratory animal models of nociception (see Table 1) have been applied to experimental neuropathic and inflammatory pain procedures, these assays generally have limited ability to reflect fully the complexity of clinical symptoms and comorbidities. Nevertheless, pain assessments in these models provide valuable tools for the mechanistic understanding of neuropathic pain syndromes and remain a key step in the discovery process for new pain medications.

Laboratory animal models have been used a variety of preclinical studies investigating cannabinoids in neuropathic pain models. The results of these studies highlight potentially important roles of the endocannabinoid system in the pathophysiology of neuropathic pain, and potential therapeutic targets to treat these conditions (Figures 1 and 2). Using complementary genetic and pharmacological approaches, distinct components of the endocannabinoid system (ie, receptors and endocannabinoid-regulating enzymes) have emerged as promising targets to treat neuropathic pain (Table 5; for additional information, Guindon and Hohmann, 2009; Rahn and Hohmann, 2009). Whether the CB₁ receptor plays a tonic role in nociception remains to

TABLE 5 Antinociceptive Effects of Cannabinoids in Preclinical Models of Neuropathic Pain

Target	Drug	Species	Anti-allodynic/Anti-hyperalgesic Stimulus Modality			Receptor Involvement (Yes/No)		Reference(s)
			Mechanical	Thermal/Heat	Cold	CB ₁	CB ₂	
Spinal nerve ligation								
CB ₁ & CB ₂	CP55,940	Mouse, rat	+			Y/N	Y/N	Sain <i>et al</i> , 2009; Scott <i>et al</i> , 2004
	WIN	Rat	+	+	+	Y	N	Bridges <i>et al</i> , 2001; LaBuda and Little, 2005; Leichsenring <i>et al</i> , 2009; Yu <i>et al</i> , 2010
CB ₂	AM1241	Mouse	+	+		N	Y	Beltramo <i>et al</i> , 2006; Hsieh <i>et al</i> , 2011; Ibrahim <i>et al</i> , 2003
	GW405833	Rat	+					Leichsenring <i>et al</i> , 2009
FAAH	URB597	Rat	+					de Novellis <i>et al</i> , 2011
Partial sciatic nerve ligation (pSNL)								
CB ₁ & CB ₂	AEA	Mouse, rat	+	+		Y	N	Desroches <i>et al</i> , 2014b; Helyes <i>et al</i> , 2003
	CP55,940	Rat	+					Fox <i>et al</i> , 2001
	HU-210	Rat	+			Y	Y	Fox <i>et al</i> , 2001; Jayamanne <i>et al</i> , 2006; Mitchell <i>et al</i> , 2005; Vuong <i>et al</i> , 2008
	WIN	Mouse, rat	+	+	+	Y/N	Y/N	Desroches <i>et al</i> , 2014b; Fox <i>et al</i> , 2001; Guindon <i>et al</i> , 2007; Gunduz <i>et al</i> , 2011; Lever <i>et al</i> , 2007
CB ₂	GW405833	Mouse, rat	+			Y	N	Li <i>et al</i> , 2017; Valenzano <i>et al</i> , 2005; Whiteside <i>et al</i> , 2005
	JWH133	Mouse	+				Y	Klauke <i>et al</i> , 2014; Yamamoto <i>et al</i> , 2008
FAAH	URB597	Mouse, rat	+	+		Y	N	Desroches <i>et al</i> , 2008, 2014b
PPAR α	PEA	Rat	+				Y	Helyes <i>et al</i> , 2003
Chronic constriction injury (CCI)								
CB ₁ & CB ₂	AEA	Rat	+	+	+	Y		Starowicz <i>et al</i> , 2012
	CP55,940	Mouse, rat	+	+		Y	N	Kinsey <i>et al</i> , 2011a; De Vry <i>et al</i> , 2004
	WIN	Mouse, rat	+	+	+	Y	Y/N	Adamson Barnes <i>et al</i> , 2016; Brownjohn and Ashton, 2012; Costa <i>et al</i> , 2004; Hama and Urban, 2004; Herzberg <i>et al</i> , 1997; Kazantzis <i>et al</i> , 2016; Lim <i>et al</i> , 2003; Linsell <i>et al</i> , 2015; Liu and Walker, 2006; Pascual <i>et al</i> , 2005; La Rana <i>et al</i> , 2008
	THC	Mouse, rat	+	+	+	Y		Kinsey <i>et al</i> , 2013; Mao <i>et al</i> , 2000; De Vry <i>et al</i> , 2004; Xie <i>et al</i> , 2016
CB ₂	AM1241	Rat	+					Wilkerson <i>et al</i> , 2012b
	AM1710	Rat	+					Wilkerson <i>et al</i> , 2012a
	GW405833	Rat	+					Brownjohn and Ashton, 2012; Hu <i>et al</i> , 2009
	JWH-015	Mouse	+	+	+		Y	Hervera <i>et al</i> , 2010
FAAH	PF-3845	Mouse, rat	+		+	Y	Y	Ghosh <i>et al</i> , 2015; Grim <i>et al</i> , 2014; Kinsey <i>et al</i> , 2010; Malek <i>et al</i> , 2016; Schlosburg <i>et al</i> , 2010
	URB597	Mouse, rat	+	+	+	Y/N	Y/N	Adamson Barnes <i>et al</i> , 2016; Caprioli <i>et al</i> , 2012; Kinsey <i>et al</i> , 2009; Russo <i>et al</i> , 2007; Starowicz <i>et al</i> , 2012, 2013; Toniolo <i>et al</i> , 2014
	URB937	Mouse	+	+		Y	N	Clapper <i>et al</i> , 2010; Sasso <i>et al</i> , 2012
MAGL	JZLI84	Mouse, rat	+	+	+	Y	Y/N	Adamson Barnes <i>et al</i> , 2016; Crowe <i>et al</i> , 2015; Ghosh <i>et al</i> , 2015; Ignatowska-Jankowska <i>et al</i> , 2015a; Kinsey <i>et al</i> , 2009, 2010, 2013; Schlosburg <i>et al</i> , 2010; Toniolo <i>et al</i> , 2014
	MJN110	Mouse	+	+		Y	Y/N	Ignatowska-Jankowska <i>et al</i> , 2015a; Wilkerson <i>et al</i> , 2016b

Table 5 (Continued)

Target	Drug	Species	Anti-allodynic/Anti-hyperalgesic Stimulus Modality			Receptor Involvement (Yes/No)		Reference(s)
			Mechanical	Thermal/Heat	Cold	CB ₁	CB ₂	
Dual FAAH & MAGL	JZL195	Mouse	+		+			Adamson Barnes et al, 2016
	SA-57	Mouse	+	+				Wilkerson et al, 2017
DAGLβ	KT-109	Mouse	+	+				Wilkerson et al, 2016a
PPARα	PEA	Mouse	+	+		Y	N	Di Cesare Mannelli et al, 2013; Costa et al, 2008
<i>Cisplatin-induced peripheral neuropathy</i>								
CB ₁ & CB ₂	AEA	Mouse	+			Y	N	Khasabova et al, 2012
	THC	Mouse	+					Harris et al, 2016
	WIN	Rat	+			Y	Y/N	Vera et al, 2013
CB ₂	AM1710	Rat	+		+	N	Y	Deng et al, 2012
	JWH133	Rat	+				Y	Vera et al, 2013
FAAH	URB597	Mouse, rat	+		+	Y	Y/N	Guindon et al, 2013; Khasabova et al, 2012
	URB937	Rat	+		+	Y	Y	Guindon et al, 2013
MAGL	JZL184	Mouse, rat	+		+	Y	Y/N	Guindon et al, 2013; Khasabova et al, 2014
<i>Paclitaxel-induced peripheral neuropathy</i>								
CB ₁ & CB ₂	CP55,940	Mouse	+		+	Y	Y	Deng et al, 2015a
	WIN	Rat	+	+		Y		Pascual et al, 2005
	THC	Mouse	+		+			Deng et al, 2015b
CB ₂	AM1241	Rat	+			N	Y	Rahn et al, 2008
	AM1710	Mouse, rat	+		+	N	Y	Deng et al, 2012, 2015b
	AM1714	Rat	+			N	Y	Rahn et al, 2008
DAGLβ	KT-109	Mouse	+					Wilkerson et al, 2016a
PPARα	PEA	Mouse	+			N	N	Donvito et al, 2016
<i>Vincristine-induced peripheral neuropathy</i>								
CB ₁ & CB ₂	WIN	Rat	+			Y	Y	Rahn et al, 2007
	AM1241	Rat	+			N	Y	Rahn et al, 2007
<i>Diabetic (streptozotocin-induced) peripheral neuropathy</i>								
CB ₁ and CB ₂	THC	Mouse, rat		+				Williams et al, 2008
	WIN	Mouse, rat	+	+		Y/N	Y	Bujalska, 2008; Doğrul et al, 2004; Ikeda et al, 2013; Jahanabadi et al, 2016; Mohammadi-Farani et al, 2010; Ulugol et al, 2004; Vera et al, 2012; Vincenzi et al, 2013
CB ₂	AM1241	Rat	+					Bujalska-Zdrożny et al, 2015; Bujalska, 2008
	JWH-015	Mouse	+	+	+		Y	Castany et al, 2016
FAAH	URB597	Rat	+	+				Hasanein et al, 2009

Table 5 (Continued)

Target	Drug	Species	Anti-allodynic/Anti-hyperalgesic Stimulus Modality			Receptor Involvement (Yes/No)		Reference(s)
			Mechanical	Thermal/Heat	Cold	CB ₁	CB ₂	
MAGL	URB937	Rat	+					Sasso <i>et al</i> , 2015
	MJN110	Rat	+					Niphakis <i>et al</i> , 2013
PPAR α	PEA	Mouse	+			Y	Y	(Donvito <i>et al</i> , 2015)
Nitroglycerin-induced migraine and hyperalgesia								
FAAH	PF-3845	Mouse	+			Y		(Nozaki <i>et al</i> , 2015)
	URB597	Mouse	+			Y		Nozaki <i>et al</i> , 2015
	URB937	Rat		+				Greco <i>et al</i> , 2015

Positive analgesic results for mechanical, thermal, and cold stimuli are denoted with a '+'. In general, the mechanical stimuli refer to von Frey filament, pressure (Randal Stiletto) or similar testing. Thermal stimuli generally included focused beams of light, hotplate plantar withdrawal or tail flick, depending on the type of neuropathy. Cold stimuli referred to positive results using plantar acetone, cold plate or similar testing. Cannabinoid (CB₁ or CB₂) receptors involvement includes results from mice lacking the gene of interest, or experiments using pharmacologic antagonists. Full or partial blockade of analgesic effects is denoted with a 'Y' (yes). Negative results are reported with an 'N' (no). Conflicting results between experiments are denoted 'Y/N' (yes/no). See listed references for more details. Anandamide; AEA. Delta-9-tetrahydrocannabinol; THC. Palmitoylethanolamide; PEA. WIN55212; WIN.

be established. However, it is noteworthy that CB₁ (–/–) mice do not display apparent altered nociception (Nadal *et al*, 2013), but rather a pain-induced increase in time spent in the dark area of the light/dark test, and decreased time and travel distance in the open area of the zero-maze apparatus. Additionally, CB₁ (–/–) mice show a reduction in sucrose preference and home cage activity (Rácz *et al*, 2015). Strikingly, selective CB₁ receptor deletion in peripheral nociceptors leads to enhanced pain behavior as well as diminished antinociceptive effects of cannabinoid receptor agonists, suggesting an important role of peripheral CB₁ receptors in neuropathic pain (Agarwal *et al*, 2007).

CB₂ receptor deletion also leads to varying effects on neuropathic pain. CB₂ (–/–) mice show exacerbated pain behavior, while CB₂ overexpression in the CNS attenuates neuropathic pain (Racz *et al*, 2008). However, other studies using knockout mice or selective inhibitors of CB₁ or CB₂ receptors show no alterations of nociceptive behavior in neuropathic pain models (Kinsey *et al*, 2009, 2010) though ceiling effects and other methodological differences between studies may have precluded the detection of enhanced nociceptive behavior. CB₁ and CB₂ receptor agonists produce antinociceptive effects in laboratory animal models of neuropathic pain (Nadal *et al*, 2013). On the other hand, the development and expression of neuropathic pain were not modified in FAAH or MAGL (–/–) mice (Nadal *et al*, 2013; Schlosburg *et al*, 2010).

This section describes the commonly used models of neuropathic pain: chronic constriction injury (CCI) of the sciatic nerve, streptozotocin (STZ)-induced diabetic neuropathy, chemotherapy-induced neuropathy, and migraine headache. Additionally, we discuss studies reporting the antinociceptive effects of various pharmacological manipulations of the endocannabinoid system in these models.

Nerve Injury Models

In rodents, one of the most common experimental approaches for inducing peripheral neuropathy is traumatic nerve injury (full or partial) via ligation, transection, or compression of the sciatic nerve (Bennett and Xie, 1988; Seltzer *et al*, 1990; Wall *et al*, 1979) or its distal branches (Lee *et al*, 2000). The partial sciatic nerve ligation model was established by Seltzer, in which approximately half of the rat sciatic nerve is ligated, generally in the proximal region of the thigh, leading to the development of mechanical allodynia and thermal hyperalgesia (Seltzer *et al*, 1990).

Another well-characterized model is chronic constriction injury (CCI), which inflicts reproducible nerve injury without complete denervation (Bennett and Xie, 1988). Originally developed in rats, one or more sutures are loosely tied around the common sciatic nerve at intervals of 1–2 mm. Rats subjected to CCI show behavioral signs interpreted as spontaneous pain, including moderate autotomy, guarding, licking and, limping of ipsilateral hind paw in addition to avoidance of placing weight on the injured side.

Within 2 days following surgery, rats show evidence of mechanical allodynia, thermal hyperalgesia and thermal allodynia in the injured paw (Bennett and Xie, 1988). Within three days of surgery, 89% of A β , 87% of A δ , and 32% of C fibers are lost, with progression to loss of 94% of myelinated fibers and 73% of unmyelinated fibers within 14 days. Electrophysiological studies have shown that primary afferents, including large-diameter myelinated fibers, spontaneously discharge at ectopic foci proximal to the injury, and these abnormal discharges may contribute to spontaneous and evoked manifestations of neuropathic pain (Munger *et al*, 1992).

As shown in Table 5, mixed CB₁/CB₂ receptor agonists, CB₁-selective receptor agonists, and CB₂ receptor-selective agonists suppress increased nociceptive behavior in the CCI, spinal nerve ligation (SNL) and partial sciatic nerve ligation (pSNL) models. In the pSNL rat model, the potency of WIN55,212-2 (0.1 mg/kg) in decreasing allodynia and hyperalgesia was significantly greater when administered daily prior to the surgery for 7 days, and for the following 2 weeks, than when administered only 2 weeks post-pSNL (Guindon *et al*, 2007). These findings suggest that activation of cannabinoid receptors can prevent the development of pain induced by nerve injury. In both SNL and pSNL models, the FAAH inhibitor URB597 evoked anti-allodynic (de Novellis *et al*, 2011), as well as anti-hyperalgesic effects (Desroches *et al*, 2008, 2014b). In addition, the fatty acid amide PEA, a substrate of FAAH and an endogenous PPAR- α ligand, produces antinociceptive effects in a rat pSNL model and in a mouse CCI model (Di Cesare Mannelli *et al*, 2013; Costa *et al*, 2008). Interestingly, daily administration of PEA in a mouse model of CCI for a week produces a long-lasting reduction of nociceptive behavior in nerve-injured animals after cessation of PEA treatment (Costa *et al*, 2008).

It is noteworthy that FAAH inhibitors produce antinociceptive effects in the CCI model, without the development of tolerance upon repeated administration (Schlosburg *et al*, 2010). In contrast, repeated administration of a high dose of the MAGL inhibitor JZL184 leads to tolerance, largely due to CB₁ receptor downregulation and desensitization (Schlosburg *et al*, 2010). However, the antinociceptive effects of a low-dose JZL184 are retained following repeated administration in a mouse model of CCI (Kinsey *et al*, 2013), suggesting that partial inhibition of MAGL is a viable therapeutic strategy to minimize the likelihood of tolerance. It is important to note that brain levels of 2-AG are approximately three orders of magnitude higher than AEA brain levels (Ahn *et al*, 2009). Thus, the disparity in tolerance development in mice treated repeatedly with a MAGL inhibitor vs a FAAH inhibitor may be related to differences in mass action of these endocannabinoids. Other possible factors contributing to the differential tolerance include differences in the efficacy of 2-AG and AEA at cannabinoid receptors and differences in CB₁ receptor-mediated circuits that these endocannabinoids activate.

The observation that selective inhibition of DAGL- β , the biosynthetic enzyme expressed predominantly on

macrophages and microglia, reduces pro-inflammatory mediators (Hsu *et al*, 2012), and reverses nociceptive behavior in a mouse model of CCI (Wilkerson *et al*, 2016a) appears to be at odds with the antinociceptive effects of MAGL inhibitors in this same model. These apparent paradoxical findings were also true in models of inflammatory pain, and can be reconciled when the dual roles of 2-AG are considered. Specifically, as 2-AG is the most highly expressed endocannabinoid in the CNS, it not only plays a major role in cannabinoid receptor activation, but also represents a rate-limiting precursor of arachidonic acid in the CNS and immune cells. Thus, inhibition of 2-AG hydrolysis or biosynthesis on macrophages and microglia results in a reduction of arachidonic acid and proinflammatory metabolites, which can reduce nociceptive behavior through cannabinoid receptor-independent mechanisms.

Streptozotocin-Induced Diabetes

Insulin-dependent diabetes is thought to cause one of the most prevalent forms of peripheral neuropathy in the developed world (Horowitz, 1993). Diabetic-induced neuropathic pain is difficult to treat and is generally unresponsive to current analgesic therapies. Genetically-based diabetic laboratory animal models include insulin deficient BB rats and NOD mice, and insulin resistant *ob/ob* and *db/db* mice. However, the most commonly used model of diabetic neuropathy consists of a single systemic injection of streptozotocin (STZ), which produces progressive and permanent degeneration of the beta cells of the pancreatic islets of Langerhans. This damage to beta cells leads to hyperglycemia, polyuria, polydipsia and weight loss (Katsilambros *et al*, 1970). STZ-induced diabetes produces consistent, long-lasting thermal and mechanical hyperalgesia, in addition to cold and mechanical allodynia (Courteix *et al*, 1993), associated with hyperexcitability of nociceptive C fibers in response to mechanical stimuli. Importantly, STZ injection elicits a time-dependent increase in the intensity of hyperalgesia, over a 4-week observation period. This time course might mimic the slow progression of painful neuropathy in individuals with diabetes from the painful stage to the degenerative and painless stage (Courteix *et al*, 1993). However, the mechanisms that produce neuropathic pain in this model are still not well understood.

Several studies have demonstrated that THC (Williams *et al*, 2008) and WIN55,212-2 (Bujalska, 2008; Doğrul *et al*, 2004; Ikeda *et al*, 2013; Jahanabadi *et al*, 2016; Mohammadi-Farani *et al*, 2010; Ulugol *et al*, 2004; Vera *et al*, 2012; Vincenzi *et al*, 2013) produce antinociception in rodent STZ-induced peripheral neuropathy. In particular, either single or repeated administration of WIN55,212-2 or AM1241, dose-dependently attenuated STZ-induced hyperalgesia (Bujalska, 2008). Furthermore, repeated administration for 1 month of medium or high doses of intranasal WIN55,212-2 improved thermal hyperalgesia and mechanical allodynia in STZ-treated mice (Toth *et al*, 2010). In addition, FAAH (Hasanein

et al, 2009; Sasso *et al*, 2015) and MAGL (Niphakis *et al*, 2013) inhibitors reduce nociceptive behavior in STZ-injected rats. Additionally, acute and repeated PEA administration evoked anti-allodynic effects in diabetic mice without the development of tolerance that required the activation of CB₁, CB₂, TRPV1, PPAR- α and PPAR- γ receptors (Donvito *et al*, 2015) (Table 5).

Chemotherapy-Induced Peripheral Neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting neurotoxic effect of chemotherapeutic agents used to treat cancer. It is a common cause for early cessation of cancer treatment and affects up to 70% of patients receiving chemotherapy (Seretny *et al*, 2014). Patients with CIPN experience symptoms of neuropathic pain such as paraesthesia, dysesthesia, allodynia, and hyperalgesia, in addition to numbness (Boyette-Davis *et al*, 2013). A wide variety of chemotherapeutic agents, including taxanes, platinum compounds, and vinca alkaloids, elicit similar neurotoxic effects in rodents and patients. These chemotherapeutic agents indirectly induce neuropathy through different mechanisms of action, which result in damage to the dorsal root ganglion satellite cells and Schwann cells that support peripheral nerves. The net effect includes structural damage, loss of peripheral nerve function, and hypersensitivity of remaining nerves. Furthermore, chemotherapeutic agents induce mitochondrial dysfunction and mitochondria-mediated oxidative stress, which lead to abnormal nerve functionality and primary afferent neuron terminal or intraepidermal nerve fiber degeneration (Han and Smith, 2013).

Paclitaxel-induced allodynia. Paclitaxel is an extremely efficacious antineoplastic agent for the treatment of solid tumors, but its significant side effects, such as neuropathy, not only decrease quality of life but also can require changes in treatment (Authier *et al*, 2009). Studies examining different paclitaxel dosing regimens in rodents have led to the development of standard protocols used in preclinical studies. A single injection of paclitaxel (32 mg/kg, intraperitoneal) in rats produces mechanical and thermal hyperalgesia, but does not elicit mechanical allodynia (Authier *et al*, 2000). On the other hand, lower doses of paclitaxel (eg, 0.5, 1, or 2 mg/kg, intraperitoneal) administered on alternate days for four injections elicit thermal and mechanical hyperalgesia as well as allodynia to light touch and cold stimuli (Polomano and Bennett, 2001). In rats, behavioral signs of neuropathic pain are accompanied by increased evoked activity and decreased conduction velocity of a subpopulation of C fibers (Dina *et al*, 2001).

It has been reported that mixed CB₁/CB₂ receptor agonists and CB₂ receptor-selective agonists are effective against paclitaxel-induced peripheral neuropathy (Deng *et al*, 2015a, 2015b; Pascual *et al*, 2005; Rahn *et al*, 2008). Notably, chronic low-dose CP55,940 suppressed paclitaxel-induced allodynia in wild-type and CB₂ (–/–) mice, but not CB₁ (–/–) mice.

By contrast, chronic administration of high-dose of CP55,940 reverses paclitaxel-induced allodynia in CB₁ (–/–) mice. However, wild-type paclitaxel-injected mice given 16 days of repeated administration of low-dose of CP55,940 showed tolerance to the anti-allodynic effects. In contrast, selective CB₂ receptor agonists produce sustained antinociceptive effects in this model without evidence of tolerance (Deng *et al*, 2015a). Specifically, repeated systemic administration of the CB₂ receptor selective agonist AM1710 suppressed paclitaxel-induced allodynia without tolerance, supporting the potential of prolonged use of CB₂ receptor agonists (Deng *et al*, 2015b). Additionally, selective inhibition of DAGL- β dose- and time-dependently reverses mechanical allodynia in paclitaxel-injected mice (Wilkerson *et al*, 2016a). Likewise, PEA elicited anti-allodynic effects in paclitaxel-treated mice in a PPAR- α -dependent manner. Repeated administration of PEA for 7 days did not produce tolerance to its anti-allodynic effects (Donvito *et al*, 2016) (Table 5).

Cisplatin-induced allodynia. Cisplatin is extensively used alone or in combination with other neurotoxic agents in the treatment of a variety of cancers, including testicular, ovarian, bladder, and lung. Repeated injections of cisplatin in rodents led to increased mechanical and thermal sensitivity (Joseph and Levine, 2009) as well as produced motor disorders (Verdú *et al*, 1999). Numerous neurophysiologic studies have shown that cisplatin decreases sensory nerve conduction velocities and reduces the amplitude of nerve action potentials (De Koning *et al*, 1987). Morphologic observations indicate that the nucleolus of primary sensory neurons is severely compromised in experimentally-induced cisplatin neuropathy (Cavaletti *et al*, 1992). Moreover, cisplatin damages myelinated nerve fibers (Boehmerle *et al*, 2014). Cisplatin may be expected to accumulate in dorsal root ganglia, leading to nuclear damage and an alteration in the peptide content, and can also exert its neurotoxic effects through Schwann cells (Yamamoto *et al*, 1997).

Cisplatin administration leads to profound alterations in the endogenous cannabinoid system. Specifically, cisplatin injections alter endocannabinoid tone, upregulating FAAH expression in the dorsal root ganglia (Guindon *et al*, 2013) (see Table 5). Drugs targeting specific components of the endocannabinoid system produce antinociceptive effects in cisplatin-induced allodynia. For example, AEA, THC, and WIN55,212-2 elicit antinociceptive effects in rodent models of cisplatin-induced peripheral neuropathy through a mechanism of action that requires activation of CB₁ (Harris *et al*, 2016; Khasabova *et al*, 2012) and CB₂ (Vera *et al*, 2013) receptors. In addition, intraplantar injection of AEA or URB597 transiently attenuated hyperalgesia through activation of peripheral CB₁ receptors. The development of cisplatin-induced hyperalgesia was delayed, and its magnitude was reduced, when daily injections of cisplatin were accompanied with URB597. This effect was mediated by the CB₁ receptor (Khasabova *et al*, 2012). Conversely, AM1710 suppressed the maintenance of mechanical and cold

allodynia in the cisplatin model through a CB₂ receptor mechanism of action, with no evidence of CB₁ receptor involvement (Deng *et al*, 2012). Finally, FAAH and MAGL inhibitors reduced cisplatin-induced mechanical and cold allodynia, which are CB₁ and CB₂ receptors-mediated (Guindon *et al*, 2013; Khasabova *et al*, 2014).

Vincristine-induced allodynia. Vincristine is prescribed to treat acute leukemia, neuroblastoma, Hodgkin's disease and other lymphomas. Vincristine arrests mitosis by binding to tubulin and blocking microtubule polymerization. In rats, 10 daily injections of vincristine (0.02, 0.1, or 0.2 mg/kg, intravenous) with a 2-day drug-free interval after the fifth day, induced hyperalgesia (Aley *et al*, 1996), and allodynia (Authier *et al*, 1999). Electrophysiological evaluations showed that vincristine caused decreased conduction velocity in myelinated and unmyelinated fibers and enhanced responsiveness of C fibers to thermal and mechanical stimuli, but did not change spontaneous activity, activation thresholds, or the number of myelinated and unmyelinated fibers (Tanner *et al*, 1998). Additionally, vincristine treatment resulted in higher firing frequency and variability in C-fibers, suggesting that alterations of activity-dependent post-synaptic effects in sensory pathways might produce a state of enhanced pain (Tanner *et al*, 1998).

Mixed CB₁/CB₂ receptor agonists and CB₂ selective receptor agonists reduce nociceptive behavior in vincristine-treated rats (Rahn *et al*, 2007) (see Table 5). In particular, systemic administration of WIN55,212-2 suppresses vincristine-evoked mechanical allodynia through a CB₁ and CB₂-dependent mechanism. In addition, AM1241 reverses vincristine-induced mechanical hypersensitivity through a CB₂ receptor mechanism of action. Spinal administration of WIN55,212-2 suppressed vincristine-evoked mechanical hypersensitivity at doses that were inactive following intraplantar administration. These effects were blocked by intrathecal co-administration of rimonabant and SR144528, implicating both CB₁ and CB₂ receptors (Rahn *et al*, 2007).

Models of Migraine Headache

Migraine is a neurological disorder characterized by recurrent debilitating attacks of headache and is the second-most common neurological disorder that accounts for more than half of the number of years lived with disability attributable to neurological diseases (Vos *et al*, 2012). Chronic migraine occurs when headaches and associated symptoms occur on more than 15 days per month, for at least 3 months, and meet diagnostic criteria for migraine with or without aura on at least 8 days per month (Headache Classification Committee of the International Headache Society (IHS), 2013). Patients with chronic migraine experience pain and other symptoms, including nausea, vomiting, osmophobia, photophobia, and phonophobia in addition to cutaneous allodynia in cranial receptive fields of the ophthalmic division of the trigeminal nerve

(Cooke *et al*, 2007; Diener *et al*, 2012). Current animal models of chronic migraine include recurrent activation of the trigeminal nociceptive system by repeated direct or indirect stimulation of the trigeminovascular and meningeal afferents through inflammatory mediators (Melo-Carrillo and Lopez-Avila, 2013) or injection of chemicals (that is, glycerol trinitrate) (Pradhan *et al*, 2014). Other models are based on genetic modifications (Estevez, 2006), and chronic alteration of the endogenous pain modulating system through serotonin depletion (Cui *et al*, 2013).

FAAH inhibitors (ie, PF-3845 and URB597) reverse allodynia in a mouse model of nitroglycerin-induced migraine through a CB₁ receptor-dependent mechanism (Nozaki *et al*, 2015). Others have found that the peripherally restricted FAAH inhibitor URB937 administration evoked anti-hyperalgesic effect in rats (Greco *et al*, 2015). Additionally, CB₂ agonism evokes an analgesic effect in a rat model of migraine (Greco *et al*, 2014) (see Table 5). Another study shows that nitroglycerin-induced hyperalgesia in rats is associated with increased activity of both FAAH and MAGL, and increased density of cannabinoid receptors binding sites in the mesencephalon. In the hypothalamus, the activity of FAAH and the density of cannabinoid binding sites is also enhanced, while in the medulla only the activity of FAAH increases (Greco *et al*, 2010). AEA also abolishes nitroglycerin-induced hyperalgesia in the phase II of formalin test (Greco *et al*, 2011). These studies suggest that a dysfunction of the endocannabinoid system may contribute to the development of migraine attacks and modulation of this system can be useful for the treatment of pain associated to migraine.

OPIOID-SPARING EFFECTS: PRECLINICAL STUDIES

Although opioids are widely accepted for the treatment of chronic pathological pain (Ballantyne and Mao, 2003), recent restructuring of prescription recommendations, particularly within the United States, strongly regulate their dosing (Dowell *et al*, 2016). These recommendations have arisen due to the epidemic of opioid misuse and abuse, as the use of opioids carries a high abuse potential (Thomas *et al*, 2015) with prescription opioid misuse preceding ~80% of new heroin users in the United States (Hedegaard *et al*, 2015). The combination of opioids with other classes of analgesics is a promising strategy to minimize abuse potential, and other opioid-related side effects (eg, sedation, constipation, physical dependence, tolerance, and respiratory depression) (Benyamin *et al*, 2008). Indeed, archaeological evidence describes the use of cannabis and opium salve for athletic injury (Bartels *et al*, 2006). Moreover, preclinical evidence demonstrates co-expression of CB₁ receptors and MORs in the periaqueductal gray and the co-administration of HU-210 and morphine into this brain region produces enhanced antinociceptive effects (Wilson-Poe *et al*, 2012, 2013). CB₁ receptors and MORs are also co-expressed within

the dorsal horn of the spinal cord (Desroches *et al*, 2014a; da Fonseca Pacheco *et al*, 2008). Of importance, these receptors share similar signal transduction pathways (Rios *et al*, 2006). Accordingly, emerging preclinical evidence shows that activation of cannabinoid receptors, via direct actions by CB₁/CB₂ agonists, or via MAGL and/or FAAH inhibition, represents a promising opioid-sparing therapeutic option for acute, inflammatory, and neuropathic pain. The reduction in opioid dose is described here as ‘opioid-sparing effects’.

Acute Pain Models

Cannabinoid receptor agonists augment the antinociceptive effects of opioids in preclinical models of acute pain, suggesting that cannabinoid agonists are opioid-sparing (Mecs *et al*, 2010; Tham *et al*, 2005; Tuboly *et al*, 2009). For example, the combination of CP55,940 and morphine synergistically attenuates thermal nociception in mice (Tham *et al*, 2005). Further, administration of a low dose of THC in combination with morphine prevents tolerance to morphine in the tail flick assay for acute pain (Cichewicz and Welch, 2003). Further studies indicate that the combination of THC and morphine produces synergistic antinociception in the tail flick assay (Cichewicz and McCarthy, 2003). In the hotplate assay for antinociception, pretreatment with CP55,940 (0.1 mg/kg), a dose that is ineffective by itself, produces a leftward shift in the dose-response of morphine (Miller *et al*, 2012). Additionally, CP55,940 produces a leftward shift of the morphine dose-response curve in the acetic acid abdominal stretching model of visceral pain in mice (Miller *et al*, 2012). Similarly, combination of URB597 and morphine produces additive antinociceptive effects in the acetic acid abdominal stretching assay and depressed wheel running in mice (Miller *et al*, 2012).

In addition to rodent models, combination of opioids and cannabinoids produces enhanced antinociceptive effects in nonhuman primates. Specifically, combined administration of morphine and THC produces augmented antinociceptive effects in the rhesus monkey warm water withdrawal test (Gerak and France, 2016; Li *et al*, 2008). Subthreshold doses of either CP55,940 or WIN55212-2 produce leftward shifts of the morphine dose-response curve in this assay, but elicit rightward and downward shifts of the morphine dose response curves in the drug discrimination and self-administration assays (Maguire *et al*, 2013), suggesting decreased abuse liability. Likewise, subthreshold doses of THC or CP55,940 produce leftward shifts in the antinociceptive dose-response relationships of fentanyl, etorphine, and buprenorphine, with increased shifts for the high-efficacy MOR agonists compared with the low-efficacy MOR agonists (Maguire and France, 2014, 2016). In contrast to work conducted on rodents (Smith *et al*, 2007), the combination of opioids and cannabinoids results in increased antinociceptive tolerance and cross-tolerance to opioids in nonhuman primates (Gerak and France, 2016), which underscores the importance of testing in higher animals. In summary, these studies provide strong evidence

that cannabinoids can augment the antinociceptive effects of opioids, which from a translational perspective could be of great benefit to reduce to opioid dosing in pain patients, though the increased rate of tolerance in nonhuman primates presents some concern.

Inflammatory Pain Models

Cannabinoid receptor agonists significantly decrease the dose of opioids needed to produce antinociception in several models of inflammatory pain, and potentially reduces opioid-induced side-effect profiles such as constipation and depressed respiration. Co-administration of THC and morphine produces synergistic antinociceptive effects in the rat CFA arthritis model (Cox *et al*, 2007). Several pieces of evidence suggest cross-talk between cannabinoids and the endogenous opioid system. For example, naloxone blocks the antinociceptive effects of THC in both arthritic and non-arthritic rats (Cox and Welch, 2004; Smith *et al*, 1998). Furthermore, THC attenuates spinal dynorphin levels in arthritic rats, indicating interactions between the opioid and endocannabinoid systems (Cox and Welch, 2004). Few studies are investigating the anatomical site of regulation of opioid-sparing effects under pathological pain conditions. Intrathecal co-administration of the endogenous opioid peptide endomorphin-1 (EM-1) and the endocannabinoid AEA reverses carrageenan-induced thermal hyperalgesia, suggesting a spinal site of action (Tuboly *et al*, 2009). Combined peripheral administration of the endogenous opioid peptide EM-1 and the endocannabinoid 2-AG attenuates mechanical allodynia produced by intra-articular injection of carrageenan, but did not affect edema (Mecs *et al*, 2010). Interestingly, a local, intraplantar co-administration of the CB₁ receptor agonist arachidonylcyclopropylamide (ACPA) with the mu-opioid receptor agonist DAMGO produces antagonistic effects on each respective compound’s antinociceptive properties in the rat CFA model of inflammatory pain (Auh *et al*, 2016). Finally, co-administration of the CB₂ receptor agonist JWH015 and morphine produces synergistic effects in the rat formalin model of inflammatory pain (Grenald *et al*, 2016).

Neuropathic Pain Models

Recent evidence supports that cannabinoids are also opioid-sparing in models of neuropathic pain. Combination of morphine and the cannabinoid receptor agonist WIN55,212 produces synergistic anti-mechanical and cold allodynia in the mouse CCI model of neuropathic pain but acts in an additive manner in the impairment of motor coordination as assessed with the rotarod assay (Kazantzis *et al*, 2016). In addition, the co-administration of the CB₂ receptor agonist JWH015 and morphine produces synergistic effects in the rat SNI model of neuropathic pain (Grenald *et al*, 2016).

The MAGL inhibitor MJN110 interacts in a synergistic manner with morphine to reverse allodynia and thermal hyperalgesia in the mouse CCI model, without opioid-

induced constipation or cannabinoid subjective effects. Importantly, these antinociceptive effects do not undergo tolerance after six days of repeated administration. CB₁ receptors, CB₂ receptors, and MORs are necessary to produce the observed antinociceptive effects (Wilkerson *et al*, 2016b). Additionally, SA-57, a dual FAAH-MAGL inhibitor that inhibits FAAH at considerably lower doses than it inhibits MAGL, interacts in an additive manner with morphine to reverse allodynia in the mouse CCI model (Wilkerson *et al*, 2017), while only producing antinociception in the tetrad assay for cannabimimetic effects. Notably, SA-57 also diminishes drug seeking behavior in mice trained to nose poke for heroin (Wilkerson *et al*, 2017).

Potential Underlying Mechanisms

There are several potential underlying neuronal and immunological mechanisms for the opioid-sparing actions of cannabinoids. One intriguing explanation accounting for the effects produced by CBD is that it may act as a positive allosteric modulator of opioid receptors (Kathmann *et al*, 2006). In addition to the above described influence of the CB₁ receptor on neuronal dynorphin release, CB₁ receptors and MORs form heterodimers, which account for enhanced neuronal antinociceptive interactions of cannabinoids and opioids (Rios *et al*, 2006). In the periphery, CB₂ receptor agonists lead to endorphin release from keratinocytes (Ibrahim *et al*, 2005). Moreover, an overwhelming amount of evidence demonstrates that within the CNS, opioid administration leads to the activation of microglia via a toll-like receptor (TLR)-4-dependent mechanism, and produces paradoxical aggravation of proinflammatory immune responses (Hutchinson *et al*, 2007; Watkins *et al*, 2009). Although speculative, CB₂ receptor agonists may reduce these proinflammatory actions of opioids, thus further enhancing antinociceptive effects. Specifically, it has been found that, within the CNS, CB₂ receptor agonists increase the anti-inflammatory cytokine IL-10, decrease IL-1 β (Wilkerson *et al*, 2012a, 2012b), decrease signaling within the AKT-ERK1/2 pathway (Merighi *et al*, 2012) and reduce mRNA of monocyte chemoattractant protein 1 (MCP-1)/CCL2 (Deng *et al*, 2015a).

In summary, opioid-cannabinoid combinations lead to enhanced antinociception in laboratory animal pain models but do not increase untoward side effects of opioids, such as respiratory depression, abuse liability (Gerak and France, 2016) or constipation (Wilkerson *et al*, 2016b). Overall, these results indicate that the actions of either direct CB₁/CB₂ receptor agonists or the inhibition of endocannabinoid degradative enzymes are a novel therapeutic avenue to decrease the doses of opioids needed for clinical pain control, and warrants further clinical investigation. Indeed, a recent meta-analysis study reported strong evidence that cannabinoids produce opioid-sparing effects in preclinical studies, but only a single study of nine clinical studies provided evidence of opioid-sparing effects in humans (Nielsen *et al*, 2017).

CANNABINOIDS AND CLINICAL MANAGEMENT OF PAIN

A comprehensive meta-analysis shows that medicinal cannabis and cannabinoids effectively alleviate different types of pain, such as neuropathic, fibromyalgia, multiple sclerosis, cancer, diabetic neuropathy, refractory pain due to multiple sclerosis or other neurological conditions, rheumatoid arthritis, noncancer pain, central pain, musculoskeletal problems, and chemotherapy-induced pain (Whiting *et al*, 2015). Here we highlight relatively recent studies examining the effectiveness of cannabinoids in the clinical management of pain as a number of comprehensive other reviews have recently been published (Lynch and Campbell, 2011; Lynch and Ware, 2015; Stevens and Higgins, 2017).

In studies evaluating smoked cannabis using cigarettes with varying THC contents, significant improvements in pain were observed compared to placebo. In neuropathic pain, cannabis containing both a lower dose (1.29 % THC) and higher dose (3.53 % THC) delivered by vaporizer demonstrated a significant analgesic response (Wilsey *et al*, 2013). In a study of multiple sclerosis spasticity and pain, smoked cannabis containing 4 % THC demonstrated a significant anti-spasticity and analgesic effect compared with placebo (Corey-Bloom *et al*, 2012). A randomized, double-blinded, placebo controlled study conducted in patients with painful diabetic peripheral neuropathy using different formulation of inhaled cannabis showed a significant dose-dependent effect on spontaneous pain score and allodynia (Wallace *et al*, 2015).

The oromucosal spray Sativex that contains an equal mixture of THC and CBD (approved in Canada and European countries) demonstrated a significant analgesic effect in neuropathic pain (Serpell *et al*, 2014). In a study involving neuropathic pain in multiple sclerosis patients, Sativex reduced pain compared to placebo at 10 weeks. However, after 14 weeks, there was no difference between oral mucosal cannabis spray and placebo groups (Langford *et al*, 2013). In a pilot study of chemotherapy-induced neuropathic pain, no statistically significant difference in pain scores was found in the oromucosal-treated group, as compared to the placebo group, although five of the 16 participants reported a 2-point grade reduction of pain score (Lynch *et al*, 2014). Recently, patients with progressive multiple sclerosis treated with an oral formulation of THC reported a significant reduction of pain (van Amerongen *et al*, 2017). Notably, this formulation was well tolerated and had a stable pharmacokinetic profile.

Nabilone, a synthetic THC analogue approved by the FDA for treatment of chemotherapy-induced nausea and vomiting (Pertwee, 2012), has also been evaluated for the treatment of pain. In a study of medication overuse headache, nabilone was superior to ibuprofen in reducing daily analgesic intake and pain intensity (Pini *et al*, 2012). Furthermore, nabilone was significantly more effective than placebo in reducing pain in patients with painful diabetic neuropathy (Toth *et al*, 2012). In a study using amitriptyline as an active control

examining sleep with pain as a secondary measure in fibromyalgia, there was no significant pain reduction (Ware *et al*, 2010). However, nabilone in combination with gabapentin improved pain reporting significantly more than gabapentin alone in multiple sclerosis patients (Turcotte *et al*, 2015).

A growing body of the preclinical data suggesting that cannabinoids in combination with opioids may lead to increased analgesic effects, while producing opioid-sparing effects in studies evaluating the clinical management of pain. A double-blind study conducted in an experimental pain model involving thermal stimuli applied to normal subjects investigated whether THC potentiates the analgesic effects of morphine (Roberts *et al*, 2006). In this study, the doses of morphine or THC used alone did not elicit a significant analgesic effect, but combination of the drugs showed an enhanced analgesic effect with respect to the affective component of pain. Indeed, the management of the affective component of pain may be especially relevant to the clinical problem of chronic pain. A phase I, and phase II study examining the efficacy of THC as an adjunct to opioid therapy for the treatment of chronic pain showed that THC enhanced pain relief in patients under opioid therapeutic regimen (Narang *et al*, 2008).

Although several studies show that ‘medical cannabis’ can improve various types of pain, cannabimimetic side effects, which include drowsiness or fatigue, dizziness, dry mouth, nausea, and cognitive effects, have also been reported (Whiting *et al*, 2015). Thus, additional research is needed to improve study methodologies, including the use of standard formulations and/or dosages, the increase in the number of subjects involved, and the general determination of the safe and effective use of cannabis for the treatment of human pain.

At the present time, there is only a single published clinical report examining whether inhibition of an endocannabinoid-regulating enzyme relieves pain. In this study, the FAAH inhibitor PF-04457845 failed to relieve pain related to osteoarthritis of the knee, as compared with placebo (Huggins *et al*, 2012). As FAAH inhibitors produce reliable antinociceptive effects in preclinical osteoarthritis models (Table 4), the lack of translation may be related to species differences or masked by the expectancy effect caused by the placebo in the clinical trial, though the comparison drug naproxen significantly improved pain compared with placebo. Also, it was not explored whether PF-04457845 in combination with naproxen would have resulted in enhanced antinociceptive effects compared with naproxen alone. In light of the death and adverse events in other subjects given repeated high doses of the FAAH inhibitor BIA-102474-101 in a clinical trial (Kerbrat *et al*, 2016), it is crucial to note that this drug has multitude of off-targets, including inhibition of essential blood-clotting factors thrombin and Factor VII (van Esbroeck *et al*, 2017; Molinski *et al*, 2017), which may contributed to its toxicity. It is also important to recognize that the highly selective FAAH inhibitor PF-04457845 was well tolerated in the

patients, with no evidence of serious adverse events (Huggins *et al*, 2012).

CONCLUSIONS AND FUTURE DIRECTIONS

An overwhelming body of convincing preclinical evidence indicates that cannabinoids produce antinociceptive effects in inflammatory and neuropathic rodent pain models. Cannabinoid receptor agonists, endocannabinoid-regulating enzyme inhibitors, and other pharmacological strategies to manipulate the endogenous cannabinoids system decrease the hyperalgesia and allodynia induced in diverse inflammatory and neuropathic pain states. In particular, the endocannabinoid degradative enzymes FAAH and MAGL are attractive targets for drug development.

The results of clinical studies consistently demonstrate efficacy of cannabis and cannabinoid receptor agonists in reducing diverse neuropathic pain states in humans. However, adverse effects associated with use of ‘medical cannabis’ as well as challenges in ensuring standardized plant constituents and concerns related to inhaling pyrolysis products of smoked cannabis are large hurdles in developing the entire plant as medicine. Thus, further research is needed to investigate whether other constituents of cannabis besides THC have therapeutic efficacy (for example, CBD) as well as explore safer delivery routes than smoking. Additionally, medicinal chemistry holds great promise to develop cannabimimetic agents that lack cannabimimetic side effects by synthesizing CB₁ receptor PAMs, selective CB₂ receptor agonists, and selective inhibitors of endocannabinoid regulating enzymes and endocannabinoid transport. Undoubtedly, drug formulation will direct the development of new cannabinoid-based medications. In addition, the combination of cannabinoids with conventional analgesics (for example, opioids, NSAIDs, gabapentin) is a promising avenue by which to increase efficacy and reduce side effects. In sum, the endogenous cannabinoid system contains multiple promising therapeutic targets and provides a strong impetus to develop cannabinoid-based medications to treat inflammatory and neuropathic pain.

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