Cannabinoid receptors and pain

Katarzyna Starowicz,* Natalia Malek and Barbara Przewlocka

Activation of both cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors reduces nociceptive processing in acute and chronic animal models of pain. In addition, nociceptive processing is tonically modulated by endogenous cannabinoids (endocannabinoids, ECs). This review examines the role of cannabinoids and ECs in the brain stem–spinal pathway of pain inhibition. Preclinical studies evaluating cannabinoids in neuropathic pain management are also reviewed. Pharmacological tools modulating the interaction of cannabinoids with its receptors and the treatment of pain by the augmentation of EC levels, specifically anandamide, are discussed. Particular attention is attributed to neuropathic pain in which pharmacological manipulation resulting in EC accumulation can be protective and produce antinociception, thereby making the system an attractive therapeutic target. Finally, the therapeutic value of cannabinoids in clinical research is summarized. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

INTRODUCTION

Within the last decades, medicine based on cannabinoids has found many applications, including antiinflammatory agents and analgesics. These achievements are particularly important for neuropathies that are refractory to conventional treatments. Cannabinoid receptors are a class of cell membrane receptors, which are a part of the G protein-coupled receptor (GPCR) superfamily.[1] There are at least two types of cannabinoid receptors, CB1 and CB2. Ligands activating these GPCRs include phytocannabinoid Δ9-tetrahydrocannabinol (Δ9-THC), numerous synthetic compounds, and endogenous compounds known as endocannabinoids (ECs). Current data indicate that cannabinoid receptor ligands undergo orthosteric or allosteric interactions with non-CB1 and non-CB2-established GPCRs, de-orphanized receptors such as GPR55, ligand-gated ion channels, transient receptor potential (TRP) channels, and other ion channels or peroxisome proliferator-activated nuclear receptors.[2–7]

In addition, cannabinoids can be divided into four different groups. The first group includes the classical cannabinoids, which consist of natural cannabinoids (such as THC) and synthetics (such as HU-210). The second group consists of nonclassical cannabinoids, whose main representative is CP-55,940, which is a nonspecific cannabinoid receptor agonist. The third class consists of aminoalkylindoles, which are synthetic cannabinoids such as AM1241. Diarylpyrazoles form the fourth group, which collectively consists of specific cannabinoid receptor antagonists, where SR141716A is a representative. This review will highlight the fifth group, i.e., the ECs.[8]

The ECs have a central nervous system (CNS) origin and include anandamide (AEA), 2-arachidonoylglycerol (2-AG), noladin ether, virodhamine and *N*-arachidonylodopamine (NADA). They do not accumulate in the cell and when released, they are synthesized *de novo* when needed. In addition, the duration of their action is short due to efficient enzymatic degradation, where they function with fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MAGL). Other enzymes involved in EC degradation include cyclooxygenase-2 (COX-2) and lipoxygenase 12/15 (LOX-12/15).[9,10]

Cannabinoids and components of the EC system have been previously summarized above. For additional detailed information, please refer to previous chapters in this review series.

*Correspondence to: starow@if-pan.krakow.pl

Department of Pain Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

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Nevertheless, a number of pharmacological tools enabling the manipulation of the release, removal, and interaction of cannabinoids with its receptors are substantial and in this review, we highlight the therapeutic preclinical and clinical studies of these pain compounds with a particular interest in neuropathic pain.

CANNABINOIDS IN PAIN PATHWAYS

The manifestation of pain and its modulation is mediated by ascending and descending pathways. Neurons in the ascending pain pathway receive input from peripheral primary afferent fibers and project from the dorsal horn of the spinal cord to a number of supraspinal sites. Two major ascending pain pathways in mammals are the spinothalamic and spinoparabrachial tracts, which encode the sensory-discriminatory and affective properties of pain, respectively. The thalamus and parabrachial nucleus receive sensory information from projection neurons in various laminae of the dorsal horn, and then relay the information to cortical and amygdalar regions, where the information is decoded as a 'painful stimulus'. The dorsal horn of the spinal cord is the location of the first synapse in pain pathway, and thus, is an attractive target for the regulation of nociceptive transmission via both local segmental and supraspinal mechanisms. The descending pathways, in turn, modulate neuronal activity in the ascending pathways, and can modulate effects on pain sensation. Interestingly, the anatomical regions involved in the modification of nociception often overlap. The midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) are the most studied regions and represent a significant system that contributes to pain modulation. The PAG is heavily interconnected to the hypothalamus and limbic forebrain structures including the amygdala and receives direct spinomesencephalic input. The PAG projects to the RVM, which in turn sends its output to dorsal horn laminae, which are important in nociceptive function. The PAG/RVM system exerts bidirectional control over nociceptive processing.[11] The neural basis of the bidirectional control of nociception can be traced to a heterogenous cell population within the RVM: OFF-cells, which are characterized by a cessation of firing during nociceptive reflexes and ON-cells, whose activity ceases abruptly just prior to the execution nociceptive reflexes. ON-cells play a pronociceptive role, which is in contrast to the activation of OFF-cells, and results in antinociception.[12,13] The PAG–RVM is recognized as

the central site of action of analgesic agents including cannabinoids.[14]

Recent studies have elucidated the role of peripheral, spinal, and supraspinal sites in CB1 receptor-dependent analgesia.[15] As previously described, the components of the EC system are found in regions involved in the transmission and modulation of nociceptive signaling (Figure 1). Behavioral tests of acute nociception and nerve injury models have confirmed that cannabinoids mediate antinociception via the activation of cannabinoid receptors. Their analgesic effectiveness was confirmed using tail-flick and hot-plate tests, observations of the animals' response to heat, or noxious stimulation models using formalin. To access the value of cannabinoids in a neuropathic pain model, mechanical and cold allodynia (pain resulting from a stimulus that ordinarily does not elicit a painful response) were measured. Hyperalgesia, which is defined as increased sensitivity to normally painful stimuli, was also an important factor in accessing the animals' sensation in neuropathic pain.

Neuroanatomical studies revealed that the CB1 receptor, which mainly functions in pain modulation, is expressed primarily in neurons of the CNS and dorsal root ganglia (DRG); however, there are also indications of their presence in tissues of nonneural origin.[16] Similarly, CB2 receptor expression is not only restricted to one tissue-type. Initially, only thought to be expressed in lymphoid tissues,[17] CB2 receptors have also been found in rodent CNS tissues as well as in human DRGs.[16]

EXOGENOUS CANNABINOIDS IN ANTINOCICEPTION

Direct support for the existence of supraspinal sites of cannabinoid antinociception was initially revealed in studies assessing the responses to acute thermal stimulation. The antinociceptive effects of Δ9-THC in a tail-flick test, which were attenuated following spinal transection, demonstrated that supraspinal sites play an important role in cannabinoid antinociceptive activity.[18] Site-specific injections of cannabinoid agonists into various brain stem regions have identified supraspinal sites of cannabinoid antinociception. Additional studies have demonstrated that microinjection of CP-55,940, WIN-55,212-2, or HU-210 into sites such as the dorsolateral PAG, dorsal raphe nucleus, RVM, amygdala, lateral posterior and submedius regions of the thalamus, superior colliculus, and noradrenergic A5 region results in antinociception.[19] Previous studies have also targeted synthetic cannabinoids at other brain stem nuclei,
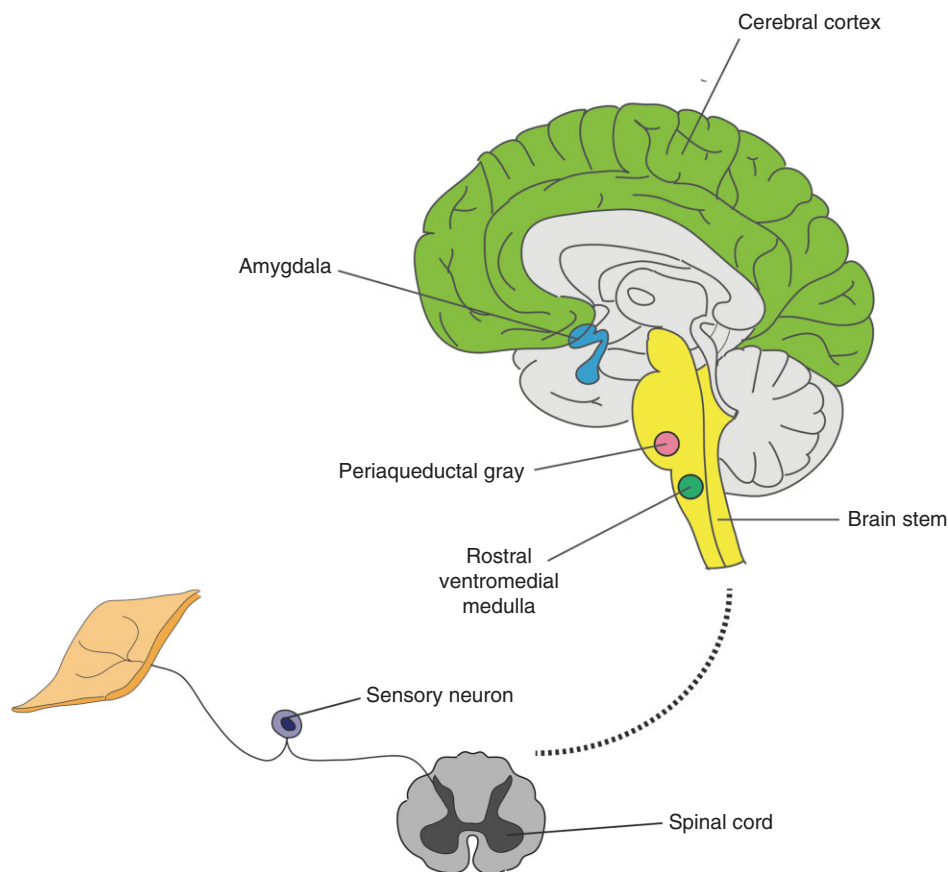
FIGURE 1 | Schematic representation of supraspinal structures involved in cannabinoid-mediated antinociception: simplified connections pattern between supraspinal structures involved in (endo)cannabinoid modulation (details in the text) of the nociceptive signal and the spinal cord.

such as the RVM, for a better characterization of cannabinoid-mediated antinociception sites. Walker's group demonstrated that site-specific administration of cannabinoids (WIN-55,212-2 and HU-210) in the RVM produced antinociception in a tail-flick test.[20] The functional role of the CB1 receptor was evident because the antinociceptive effects of HU-210 were blocked using the potent CB1 receptor antagonist, rimonabant. Moreover, the receptor-inactive enantiomer WIN-55,212-3 failed to induce antinociception after microinjection into the same site.[20] Electrophysiological studies have provided functional insight into the mechanism mediating these antinociceptive effects. *In vivo* recordings provided direct evidence that cannabinoids modulated ON- and OFF-cells in the RVM,[21,22] thereby demonstrating the ability of these ligands to control descending pain modulatory signaling via a process similar to that of morphine. Cannabinoids increased ongoing OFF-cell activity and reduced both the OFF-cell pause and the ON-cell burst that occurs just prior to the tail-flick reflex—activities that are mediated by a CB1 receptor-mediated mechanism.[22] Pharmacological inactivation of RVM

with site-specific administration of the GABA-A receptor agonist muscimol blocked the antinociceptive effects, but not the motor deficits of systemically administered WIN-55,212-2,[22] which revealed a GABAergic link in cannabinoid antinociceptive mechanisms. At the cellular level, cannabinoids exert their physiological effects in the RVM via presynaptic inhibition of GABAergic neurotransmission.[23] Taken together, these results suggest that nociceptive responsiveness is modulated in the RVM by ECs.

Lichtman et al. demonstrated that the administration of CP-55,940 in the vicinity of the posterior ventrolateral PAG/dorsal raphe, but not in the caudate putamen, produced antinociception, catalepsy, and hypothermia, which was specific for the active stereoisomer.[24] Microinjection of another cannabinoid HU-210 into the dorsal PAG also produced a CB1 receptor-mediated suppression of formalin-evoked nociceptive behavior.[25] Moreover, exogenous cannabinoids modulated ultrasound-induced aversive responses in rats via effects on the dorsal PAG. These effects may be partly mediated by the dorsal PAG, but cannot be explained only by CB1 receptor activity

because these effects were insensitive to blockade by rimonabant, a specific CB1 receptor antagonist.[26]

ROLE OF ECs IN PAIN MODULATION

Electrical stimulation of the dorsal and lateral PAG resulted in cannabinoid receptor-mediated analgesia, which was insensitive to blockade by opioid antagonists and was blocked by intra-PAG microinjection of the cannabinoid antagonist, rimonabant. The effect was concurrent with the mobilization of AEA.[27] Consecutive studies demonstrated that 2-AG and AEA were elevated in dorsal midbrain regions containing the entire PAG, concomitantly with the expression of nonopioid stress-induced analgesia (SIA). Exposure to a 3-min continuous foot shock induced a CB1 receptor-mediated SIA independent of endogenous opioids.[9] Moreover, microinjection of FAAH inhibitors such as URB-597 and arachidonoylserotonin (AA-5HT)[28] also enhanced SIA in a CB1 receptor-dependent manner. Microinjection of the FAAH inhibitor URB597 into the ventrolateral PAG has been reported to elevate EC levels (both AEA and 2-AG) and induce biphasic effects on thermal nociception via the activation of CB1 and TRPV1 (transient receptor potential vanilloid type 1) receptors.[14] In this study, TRPV1-mediated antinociception and CB1 receptor-mediated nociception induced by URB-597 correlated with the enhanced or reduced activity of RVM OFF-cells, suggesting that these effects occurred via stimulation or inhibition of excitatory PAG output neurons, respectively. However, at the highest dose tested, URB-597 (4 nmol/rat) and WIN-55,212-2 (25–100 nmol/rat), only demonstrated a CB1 receptor-mediated analgesic effect, which correlated with stimulation of RVM OFF-cells. Thus, AEA (but not 2-AG) may affect the descending pathways of pain control by acting at either CB1 or TRPV1 receptors in selected PAG subregions.[14] Microinjection of the MAGL inhibitor URB-602 into the PAG also induced a CB1 receptor-mediated enhancement of stress antinociception and specifically elevated levels of 2-AG (but not AEA) in this region.[9] These data showed a physiological role for endogenous 2-AG in pain modulation at the level of the midbrain PAG. However, not all of the effects of ECs were mediated by CB1 receptors, and thus, it is important to demonstrate that EC activity may be blocked by specific cannabinoid antagonists.

Another brain structure where the role of ECs has been demonstrated is the amygdala, where itformalin-evoked nociceptive behavior[25] mainly coordinates fear and defensive responses. The highest CB1 receptor mRNA expression was found in the basolateral nucleus of the amygdala (BLA), a structure that has been reported to be involved in the modulation of acute or tonic nociceptive processing.[29] The antinociceptive effects of WIN 55,212-2 were demonstrated in both tail-flick and formalin tests. Rats demonstrated a dose-dependent increase in the latency to withdraw from a thermal noxious stimulus in a tail-flick test and a decrease in formalin-induced pain behaviors. These effects were attenuated in the presence of the specific CB1 receptor antagonist AM251. In contrast, the CB2 receptor antagonist SR144528 exhibited no effect on antinociception produced by WIN 55,212-2, suggesting that the antinociceptive actions of WIN 55,212-2 were mediated by CB1 receptor. Moreover, bilateral lesions of the amygdala rendered rodents less sensitive to the antinociceptive effects of the potent synthetic cannabinoid WIN-55,212-2.[30] Furthermore, FAAH and MAGL are expressed on the postsynaptic and presynaptic sites, respectively, in the basolateral and lateral amygdala,[31–33] indicating the existence of mechanisms for the deactivation of AEA and 2-AG therein. Both conditioned[34] and unconditioned[35] SIA are dependent upon proper amygdala function. These observations together with the demonstration of cannabinoid-mediated antinociception (WIN-55,212-2 microinjection into amygdala)[19] suggest that ECs may naturally serve to suppress noxious stimuli and pain via actions in the amygdala.

Early observations of the antinociceptive properties of cannabinoids have laid a foundation for further research providing a hope that modulation of the EC system may have an effect on chronic pain. Because there is no current proven treatment to cure neuropathic pain, we will focus on cannabinoid-based treatment options for neuropathic pain.

CANNABINOIDS IN NEUROPATHIC PAIN

Neuropathic pain is one of the most challenging ailments with respect to understanding pain mechanisms and rationalizing approaches for the treatment of pain. An effective and safe neuropathic pain treatment still remains as a large unmet therapeutic need. One of the emerging approaches to attenuate hyperexcitability in pain circuitry is to enhance the cellular inhibitory mechanisms by targeting cannabinoid receptors.

The most commonly used experimental models of neuropathic pain are on the basis of injury of the sciatic nerve, which is caused by a chronic constriction injury (CCI),[36] partial ligation of the sciatic nerve,[37] or L5 and L6 spinal nerve ligation (SNL).[38] All of these

models produce mechanical and thermal allodynia and hyperalgesia ipsilateral to the site of the injury.

Receptor-Mediated Analgesic Mechanisms

Initial reports describing the effectiveness of WIN-55,212-2, a high affinity cannabinoid agonist for neuropathic pain treatment, were reported by Herzberg et al.[39] CCI-induced hyperalgesia and allodynia were alleviated upon WIN-55,212-2 administration and were counteracted using the CB1 receptor antagonist/inverse agonist SR141716A. Similarly, the analgesic properties of WIN-55,212-2 were demonstrated in an SNL model in a CB1 receptor-dependent manner. Signs of painful neuropathy were reversed on the ipsilateral site without affecting the sensory thresholds of the contralateral paw.[40] Another, albeit relatively smaller, series of studies have examined the effect of prolonged treatment with cannabinoids under neuropathic pain conditions. As previously reported by Costa et al., repeated treatment with WIN-55,212-2 inhibits thermal and mechanical hyperalgesia. Moreover, daily treatment over this period did not induce tolerance against the antihyperalgesic and antiallodynic effects. Repeated treatment with WIN-55,212-2 was shown to be effective in reducing the effects of inflammatory mediators, which are known to sensitize the peripheral sensory nerve endings, resulting in hyperalgesia. Plasma prostaglandin E2 (PGE2) and nitric oxide (NO) levels were reduced to control values following chronic WIN-55,212-2 treatment,[41] which highlights the effectiveness of WIN-55,212-2 in alleviating not only neuropathic pain, but also in peripheral inflammatory conditions. This is an important study in light of the therapeutic potential of cannabinoids, which was able to mitigate neuropathic pain.

In addition to WIN-55,212-2, several other mixed cannabinoid receptors agonists (CP-55,940 and HU-210) have been shown to suppress neuropathic pain in the CCI model, primarily via CB1 receptor-mediated mechanisms. WIN-55,212-2, CP-55,940, and HU-210 produced a reversal of mechanical hyperalgesia.[42] These data indicated that cannabinoid receptor agonist activity is involved in cannabinoid receptor anatomical localization. Only peripheral (subcutaneous, s.c.), and not central (intrathecal, i.t.), administration of the CB1 receptor antagonist SR141716A blocked the antihyperalgesia induced by WIN-55,212-2. Thus, a peripheral site of action for this effect was proposed. Studies from the Lichtman group significantly contributed to the development of receptor-specific compounds that selectively activate the CB2 receptor without eliciting CB1 receptor-mediated cannabimimetic effects, such as locomotor inhibition and hypothermia. Kinsey et al.[43] reported that the ethyl sulfonamide Δ9-THC analog O-3223 displayed specificity and efficacy for the CB2 receptor. Subsequently, O-3223 was evaluated in a variety of murine pain models including CCI-induced neuropathic pain and was reported to reduce thermal hyperalgesia. Antihyperalgesic effects of O-3223 were blocked by pretreatment with the CB2 receptor-specific antagonist SR144528, but not by the CB1 receptor antagonist rimonabant. Moreover, unlike CP-55,940 (both cannabinoid receptors agonist), O-3223 did not elicit acute antinociceptive effects in a hot-plate test, hypothermia, or motor disturbances, as assessed in the rotarod performance test. O-3223 did not affect basal nociception or elicit overt behavioral effects. This novel Δ9-THC analogue exhibited significant antiinflammatory and antinociceptive effects *in vivo*, but did not cause any CB1 receptor-specific behavioral effects observed with a cannabinoid agonist such as CP-55,940. Thus, this compound serves as a model molecule for the development of CB2 receptor agonists with increased antinociceptive potency. The benefit of specifically using cannabinoid activity on the CB2 receptor over mixed cannabinoid receptors agonists is the lack of central side effects, which offers a promising alternative for future analgesic therapy. Several reports have demonstrated the contribution of CB2 receptor-specific agonists in the suppression of CCI-induced mechanical allodynia,[44] although pharmacological specificity has not yet been consistently evaluated. However, it is remarkable that CB2 receptor mRNA is upregulated in the lumbar spinal cord following CCI[45] primarily in nonneuronal cells. Moreover, tolerance failed to develop after repeated administration of the CB2 receptor-specific agonist A-836,339, highlighting its therapeutic benefits, namely the suppression of hyperalgesia and allodynia in the absence of side effects.[46] Recent work by Leichsenring et al. analyzed the effect of repeated administration of the CB2 receptor agonist GW-405,833 on mechanical allodynia in the spinal cord and compared these effects to the synthetic cannabinoid WIN-55,212-2. Both drugs were applied daily in a low nonpsychotropic dosage and showed an equal effectiveness in reducing mechanical allodynia induced by SNL. Chronic administration of WIN-55,212-2 produced antiallodynic effects up to 6 days following the final injection. A reappearance of glial activation was also associated with the return of neuropathic pain features in this study.[47] An overview summarizing the outcome of preclinical studies using CB1-/CB2-receptor ligands is presented in Table 1.

TABLE 1 | Summary of Research Targeting CB1- and CB2-R and Their Actions on Pain Behavior

Exogenous Cannabinoid	Influence on Endocannabinoid System	Effect in Pain	References
A-836,339	CB2-R agonist	CB2-R-mediated antihyperalgesic effect in Freund's adjuvant model of inflammatory pain; analgetic in the CCI, skin incision, and capsaicin-induced hyperalgesia models	46
AM-404	CB1-/CB2-R partial agonist; inhibitor of anandamide reuptake	Reversion of hyperalgesia and enhancement of serotonergic activity in the CCI model; reduction of Fos-immunoreactivity in the spinal cord of neuropathic rats	49,50
CP-55,940	CB1-/CB2-R agonist	Antinociception after PAG microinjection	24
GW-405833	CB2-R agonist	Reduction of mechanical allodynia and glial activation in long-term treatment in neuropathic rats	47
HU-210	CB1-/CB2-R agonist	Reduction of formalin-evoked nociceptive behavior and Fos-protein expression after dorsal PAG administration; reversion of mechanical hyperalgesia in tail-flick, catalepsy, rotarod, and hypothermia tests; elevation of tail-flick latency after RVM microinjection in SNL model	19,25,42
JZL184	MAGL inhibitor	Attenuation of mechanical and cold allodynia after CCI of the sciatic nerve	51
O-3223	CB2-R agonist; ethyl sulfonamide THC analog	Reduction of nociceptive behavior in the formalin test, reduction of thermal hyperalgesia in the CCI of the sciatic nerve model and reduction of edema and thermal hyperalgesia elicited by intraplantar injection of Lipopolysaccharides (LPS)	43
OL-135	Reversible FAAH inhibitor	Decrease of mechanical allodynia and acetone-induced cold allodynia in mice CCI model	51
PF-3845	FAAH inhibitor	Attenuation of mechanical and cold allodynia in wild-type mice in CCI model	52
SR141716A (rimonabant)	CB1-R antagonist	Prevention of antiallodynic properties of WIN55,212-2 in rat SNL model; blockage of HU210-induced attenuation of increased locomotor activity; attenuation of the antinociception produced by HU210 on the tail-flick reflex; blockage of WIN 55,212-2 suppression of heat-evoked activity in chronic constriction injury (CCI) model; suppresion of antinociception after microinjection into RVM or dorsolateral PAG in SIA model	19,25,40
SR-144528	CB2-R antagonist	Prevention of antiarthritic effects of CB2-R agonists in CIA model in chronic administration	43

TABLE 1 | Continued

Exogenous Cannabinoid	Influence on Endocannabinoid System	Effect in Pain	References
URB-597	Irreversible FAAH inhibitor	Potentiation of antinociception after injection into the dorsolateral PAG in SIA model; elevation of PAG anandamide and 2-AG levels after intra-PAG injection in healthy rats; decrease of mechanical allodynia and acetone-induced cold allodynia in mice CCI model	9,14,51
URB-602	MAGL inhibitor	Elevation of 2-AG level in the dorsal midbrain and enhancement of antinociception in SIA model	9
WIN-55,212-2	CB1-/CB2-R agonist	Alleviation of thermal and mechanical hyperalgesia in CCI model; effectivness against thermal hyperalgesia and mechanical allodynia in SNL model; elevation of tail-flick latencies after injections into the amygdala, the lateral posterior and submedius regions of the thalamus, the superior colliculus, the noradrenergic A5 region, periventricular structures and RVM; production of analgesia by modulating RVM neuronal activity; leading to analgesic effect in the plantar test after VL-PAG injection; analgesic effect to noxious stimulus, lower in animals with bilateral amygdala lesions than control; increase of withdrawal thresholds to cold, mechanical and noxious thermal stimulation in CCI model; reduction of thermal hyperalgesia, revertion of mechanical allodynia and reduction of firing activity after chronic administration in CCI model; reduction of tactile allodynia and spinal cord glial activation together with expression of the cathepsins X and S in SNL model; suppression of heat-evoked activity, decrease of the receptive field areas of dorsal horn WDR neurons and reversion of the hyperalgesia in CCI model	14,19,22,30,39,40,42,47,49

CCI, chronic constrain injury; PAG, periaqueductal gray; SNL, spinal nerve ligation; SIA, stress-induced analgesia; CIA, collagen-induced arthritis; RVM, rostral ventromedial medulla

Discrepant and unexpected results appeared with regard to the CB1 receptor-specific antagonist SR141716A, which when administered acutely, is prohyperalgesic and proallodynic in a CCI model.[39] However, when administered chronically, SR141716A reduces thermal and mechanical hyperalgesia in both rats and CB1 receptor (+/+) mice, but fails to produce an effect in CB1 receptor (−/−) mice.[48] Recent reports on the anti- or pronociceptive profile of cannabinoid receptor antagonists were ambiguous and require further studies.

Increasing EC Concentration for Pain Relief

CCI increases AEA and 2-AG levels in the PAG and RVM, which are sites that have been implicated in the descending modulation of pain.[53] CCI also increases the levels of endogenous AEA, but not 2-AG, in the dorsal raphe, observations that may help to explain the antihyperalgesic efficacy of the AEA transport inhibitor in this model. CCI results in elevated levels of serotonin (5-HT) in the dorsal raphe, which can be suppressed by both WIN-55,212-2 and AM404 in a CB1 receptor-dependent manner.[49] CCI-induced Fos expression was also observed in response to nonnoxious mechanical stimulation in the dorsal superficial laminae of the lumbar spinal cord. Chronic administration of AM404 significantly decreased CCI-induced Fos expression in this region via cannabinoid receptors and TRPV1-mediated mechanisms.[50]

An inhibitor of EC cellular reuptake AM404 increased the accumulation, and as a consequence, the bioavailability of ECs via a mechanism that is not clearly understood. AM404 normalizes CCI-induced changes in NO activity,[54,55] cytokine levels (e.g., TNF-α and IL10), and NF-κB levels.[54] In CCI rats, chronic administration of AM404 suppressed plasma extravasation.[56] AM404 may be a good target candidate for neuropathic pain treatment because it does not affect locomotor behavior when administered chronically or acutely,[54] which is a main side effect associated with the direct activation of the CB1 receptor. However, antihyperalgesic effects observed with AM404 are likely to be dependent not only on cannabinoid receptors, but also on TRPV1. As reported by Rodella et al.,[50] AM404 significantly reduced Fos expression, a marker of activated neurons, in neuropathic animals. However, co-administration of cannabinoid receptors and TRPV1 antagonists reduced the effect of AM404. Related studies have reported that the chronic administration of synthetic analogs of plant cannabinoids, which were effective in alleviating hyperalgesia independent of
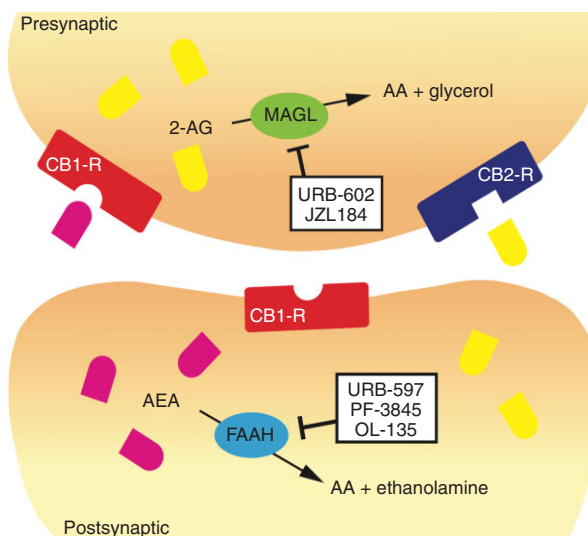

FIGURE 2 | Schematic representation of a promising strategy to treat neuropathic pain syndromes by targeting the main EC degradation enzymes. Anandamide (AEA) is hydrolyzed into arachidonic acid (AA) and glycerol primarily by fatty acid amide hydrolase (FAAH) in the postsynaptic neuron. 2-Arachidonoylglycerol (2-AG) is hydrolyzed into AA and ethanolamine primarily by monoacylglycerol lipase (MAGL) in the presynaptic neuron, although other 2-AG hydrolases (localized postsynaptically) may also participate in this process. Both FAAH (URB-597; PF-3845 and OL-135) and MAGL inhibitors (URB-602 and JZL184) reduce neuropathic pain symptoms.

cannabinoid receptors, was attenuated by the TRPV1 antagonist capsazepine.[57,58]

An alternative approach in the utilization of endogenous cannabinoid systems is the inhibition of FAAH and MAGL enzymes (Figure 2). Supporting data demonstrated that FAAH (−/−) mice[59] or mice treated with FAAH inhibitors, such as URB597 (the irreversible FAAH inhibitor) and OL-135 (the reversible FAAH inhibitor), demonstrated significantly elevated levels of AEA in brain structures and increased pain threshold in pain models.[60] OL-135 and URB597 attenuated cold and mechanical allodynia in a mice CCI model, which was dependent upon the activation of both CB1 and CB2 receptors. In addition, both OL-135 and URB597 were antinociceptive in FAAH (+/+) mice, but failed to produce such an effect in FAAH (−/−) mice.[59] JZL184, which is a novel MAGL inhibitor, attenuated CCI-induced mechanical and cold allodynia via indirect activation of the CB1 receptor. Moreover, MAGL was efficacious in attenuating neuropathic nociception in both FAAH (+/+) and FAAH (−/−) mice.

Studies performed on the recently developed FAAH inhibitor PF-3845 showed an attenuation of CCI-induced mechanical and cold allodynia in

wild-type mice.[51] PF-3845 offers many advantages over previous FAAH inhibitors, such as an increased FAAH specificity and longer duration of *in vivo* activity.[52] FAAH inhibition did not elicit antiallodynic effects in CB1 receptor (−/−) or CB2 receptor (−/−) mice, indicating that both receptor subtypes are necessary for the expression of these effects. However, when investigating the antiallodynic effects of MAGL inhibition, it was reported that inhibition of 2-AG, which is the main catabolic enzyme, prevented its antiallodynic effects in CB1 receptor (−/−), but not in CB2 receptor (−/−) mice, indicating that the activity of 2-AG was driven by a CB1 receptor-specific mechanism. Thus, it appears that AEA and 2-AG elicit antiallodynic effects via distinct cannabinoid receptor mechanisms of action. Several studies have postulated[52] that the elevation of the endogenous levels of AEA produces antiallodynic effects via the activation of both the CB1 and CB2 receptors. In contrast, these data suggest that CB1 receptors are necessary for the antiallodynic effects resulting from elevated levels of 2-AG; however, CB2 receptors were dispensable. Recently, we reported a significant reduction in neuropathic pain symptoms following inhibition of the AEA hydrolytic enzyme with URB597 in a rat CCI model.[61] Depending on the dose of URB597 used, and on the consequential elevation of endogenous AEA levels (lesser or higher), analgesia was mediated via CB1 or TRPV1 receptors, respectively, which was also dependent on its local concentration. These data suggest that both the indirect modulation of TRPV1 function as well as the strengthening of endogenous AEA signaling by inhibition of its enzymatic degradation hold promise for the development of novel multitarget pharmacological treatments.

Activation of cannabinoid receptors and pharmacological manipulation of EC accumulation or breakdown suppresses neuropathic nociception in rodents. Both FAAH and MGL represent potential therapeutic targets for the development of pharmacological agents to treat chronic pain resulting from nerve injury.

SUMMARY

The preclinical reports presented in this review support cannabinoid use in pain that is resistant to conventional treatment. Trials on the analgesic properties of cannabinoids have now entered the clinic, e.g., studies on *Savitex*. *Savitex* is comprised of Δ9-tetrahydrocannabinol and cannabidiol (Δ9-THC and CBD), and has been shown to reduce allodynia in patients suffering from pain of neurological origin, which was often refractory to other treatments.[62] Moreover, *Savitex* was successfully used in patients with advanced cancer-associated pain, who were immune to the chronic administration of opioids.[63] Furthermore, *Savitex* was shown to be well tolerated and patients exhibited no severe adverse effects in the nervous system. Patients also experienced pain relief, which was maintained without drug-related toxicity or dose escalation. Recent clinical trials reported that *Sativex* had a negligible effect on abuse potential and minimal potential psychoactive effects.[64]

Scientific articles reviewed herein demonstrated the ability of cannabinoids to treat pain; however, cannabinoid compounds are more effective in the context of chronic pain compared to the management of acute pain. Recently, an interesting finding by Pernia-Andrade et al.[65] may partially explain why, in human trials, drugs targeting the cannabinoid system have been negative for the treatment of most types of acute and postsurgical pain, but were effective for some chronic pain states. Studies have demonstrated that cannabinoid drugs and ECs generated in the spinal cord weaken the inhibitory control of pain-sensing neurons, thereby opening a 'pain gate' that enhances the neurotransmission of both painful and nonnoxious mechanical stimuli via 'pain pathways' to higher centers in the brain. The pain-promoting activity of ECs fades during the development of chronic pain, which is induced by inflammation or nerve injury. An increasing number of laboratory studies have demonstrated an increased attenuation of pain resulting from the administration of cannabinoids. Recent developments have focused on the EC system as an integral component of pain control. Most drugs acting specifically on peripheral cannabinoid receptors and inhibiting FAAH/MAGL enzyme activity to prevent the breakdown of ECs offer a potential ability to separate the beneficial analgesic effects from the unwanted drug side effects and indicate a promising role for EC-based medicines in pain management.

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