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The Endocannabinoid System and Pain

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Abstract

The therapeutic potential of cannabinoids has been the topic of extensive investigation following the discovery of cannabinoid receptors and their endogenous ligands. Cannabinoid receptors and their endogenous ligands are present at supraspinal, spinal and peripheral levels. Cannabinoids suppress behavioral responses to noxious stimulation and suppress nociceptive processing through activation of cannabinoid CB_1 and CB_2 receptor subtypes. Endocannabinoids, the brain's own cannabis-like substances, share the same molecular target as Δ^9-tetrahydrocannabinol, the main psychoactive component in cannabis. Endocannabinoids serve as synaptic circuit breakers and regulate multiple physiological and pathological conditions, e.g. regulation of food intake, immunomodulation, inflammation, analgesia, cancer, addictive behavior, epilepsy and others. This review will focus on uncovering the roles of anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the two best characterized endocannabinoids identified to date, in controlling nociceptive responding. The roles of AEA and 2-AG, released under physiological conditions, in modulating nociceptive responding at different levels of the neuraxis will be emphasized in this review. Effects of modulation of endocannabinoid levels through inhibition of endocannabinoid hydrolysis and uptake is also compared with effects of exogenous administration of synthetic endocannabinoids in acute, inflammatory and neuropathic pain models. Finally, the therapeutic potential of the endocannabinoid signaling system is discussed in the context of identifying novel pharmacotherapies for the treatment of pain.

Keywords

anandamide; 2-arachidonoyl glycerol; FAAH; MGL; endocannabinoid transporter; analgesia; inflammatory; neuropathic pain

INTRODUCTION

Cannabis has been used for more than twelve thousand years and for many different purposes (i.e. fiber, medicinal, recreational). However, the endocannabinoid signaling system has only recently been the focus of medical research and considered a potential therapeutic target [1–3]. Endocannabinoids mimic the pharmacological actions of the psychoactive principle of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC) [4]. Endocannabinoids are endogenous lipid-signaling molecules. They are generated in the cell membrane from phospholipid precursors and possess cannabimimetic properties because they bind and activate one or more cannabinoid receptor subtypes [5,6]. Endocannabinoids are implicated in different physiological and

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CONFLICT OF INTEREST
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pathological functions (regulation of food intake, immunomodulation, inflammation, analgesia, cancer, addictive behavior, epilepsy and others) [2,7]. The two best-studied endocannabinoids isolated to date are arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). AEA is hydrolyzed by the enzyme fatty-acid amide hydrolase (FAAH) whereas 2-AG is degraded by the enzyme monoacylglycerol lipase (MGL) [7,8]. The main goal of this review will be to uncover the role of AEA and 2-AG in pain modulation. This will be accomplished by reviewing studies examining mobilization of endocannabinoids under physiological conditions or by using pharmacological tools that inhibit their uptake or degradation. This review will also consider studies employing exogenous administration of synthetic endocannabinoids in combination with other pharmacological approaches aimed at regulating their uptake or degradation. The overall goal is to understand the physiological role of the endogenous ligands at different levels of the pain pathway and in different models of pathological pain.

CANNABINOID RECEPTOR PHARMACOLOGY

Cannabinoids produce their effects through the activation of distinct G protein-coupled receptors identified as the cannabinoid CB_1 [9,10] and CB_2 receptors [11]. Cannabinoid CB_1 and CB_2 receptors are members of the superfamily of seven-transmembrane-spanning G protein-coupled receptors and share 44 % identity at the protein level [11,12]. Similarity increases to 68 % when only the transmembrane region is considered [11,12]. Activation of both cannabinoid receptor subtypes inhibits adenylate cyclase activity by coupling to the α–subunit of the G protein of the $G_{i/o}$ family ($G_{i\ 1,\ 2\ and\ 3}$, and $G_{o\ 1\ and\ 2}$) [13]. In contrast to CB_2 receptor activation, CB_1 receptor activation modulates calcium or potassium conductance [14,15], properties linked to the suppression of neuronal excitability and neurotransmitter release. However, activation of MAPK and Krox-24 expression presumably through the activation of G-protein βγ subunits is another signalling mechanism recruited by both CB_1 and CB_2 receptors [16,17]. Furthermore, CB_1 receptor activation can inhibit type 5-HT_3 ion channels [18], modulate the production of nitric oxide [for review see 19,20], alter sodium channel conductance [21] and activate the Na^+/H^+ exchanger [22]. Signaling mechanisms engaged by activation of CB_1 and CB_2 receptors have been recently reviewed [13,23].

Cannabinoid CB_1 receptors are found mainly in the CNS and, to a lesser extent, in certain peripheral tissues [24]. At the peripheral level, they are localized in adrenal gland, adipose tissue, heart, liver, lung, prostate, uterus, ovary, testis, bone marrow, thymus, tonsils and presynaptic nerve terminals [12,20,25–27]. Within the brain, they are found in the cerebral cortex, hippocampus (with highest concentrations in the dentate gyrus), amygdala, basal ganglia, substantia nigra pars reticulata, internal and external segment of the globus pallidus and cerebellum (molecular layer) [15,20,24]. More significantly for the purposes of the present review, they are found at central and peripheral levels of the pain pathways [28–32]. The distribution of cannabinoid receptors provides an anatomical basis for the analgesic effects of the cannabinoids. Activation of presynaptic CB_1 receptors in different brain regions or on primary afferents inhibits the release of neurotransmitters by decreasing calcium conductance and by increasing the potassium conductance [26].

CB_2 receptors are primarily localized to cells of the immune system. CB_2 receptors are mainly found in the spleen, tonsils and thymus, tissues responsible for immune cell production and regulation [11,12,15]. These immune cells include mast cells, B cells, T4 and T8 cells, microglial cells, macrophages, natural killer cells and, to a lesser extent, monocytes and polymorphonuclear neutrophils [12,15,33]. Previous reports suggested that CB_2 receptors were absent in neurons of the central nervous system (CNS) [11,34]. However, recent studies suggest that they are found in the brain, on dorsal root ganglia, in the lumbar spinal cord, on sensory neurons, on microglia as well as in peripheral tissues [35,36].

A better understanding of the role of cannabinoid receptors in different physiological processes has been obtained through research employing pharmacological and genetic tools such as competitive antagonists and knockout mice with disrupted CB_1 [37,38] and/or CB_2 genes [39,40]. Pharmacological evidence also supports the existence of one or more additional receptors for cannabinoids distinct from CB_1 and CB_2 receptors (reviewed in [41,42]). Of particular recent interest are the GPR55 receptor [43–45] and GPR3, GPR6 and GPR12 which are sphingosine-1-phosphate lipid receptors [46–48]. More work is necessary to determine the connection of novel receptor subtypes such as GPR55 to the endocannabinoid system using more specific compounds and genetic tools.

ENDOCANNABINOIDS

The discovery of AEA [49], the first endocannabinoid isolated from brain, was followed a few years later by the identification of 2-AG [50,51]. Since then, several putative endocannabinoids have been isolated which include noladin ether [52], virodhamine [53] and N-arachidonoyldopamine (NADA) [54,55]. Much less information is known about the endocannabinoid-like properties of these latter putative endogenous ligands (see [56] for a review). Indeed, elucidation of the endogenous function of these compounds in different physiological processes and their precise mechanisms of action requires further investigation [57]. Here, we will consider the roles of different cannabinoid receptors, different endocannabinoids and the machinery responsible for their synthesis and degradation. In some cases, functions of the endocannabinoid system are surmised following pharmacological inhibition of endocannabinoid deactivation. Thus, FAAH and MGL inhibitors increase endocannabinoid accumulation (AEA and 2-AG, respectively) by inhibiting hydrolysis of fatty-acid amides and monoacylglycerols; these enzymes have multiple substrates. Both AEA and 2-AG are derivatives of arachidonic acid and bind to cannabinoid CB_1 and CB_2 receptors, although with different affinities and efficacies [58]. However, the variable affinity for cannabinoid receptors may be due, in part, to the existence of distinct binding sites for the different ligands on cannabinoid receptors, as documented by molecular modeling studies [59].

SYNTHESIS AND RELEASE OF AEA AND 2-AG

Endocannabinoids are produced on demand either by activity-dependent or receptor-stimulated cleavage of membrane phospholipid precursors. Endocannabinoids can be released immediately from cells after their production since they are highly lipophilic and thus are poorly suited for storage (for review see [8,60,61]). Endocannabinoid signaling is regulated by synthesis, release, uptake and degradation. Membrane depolarization, increases in intracellular calcium levels and receptor stimulation can all activate enzymatic processes leading to the cleavage of membrane phospholipids precursors and subsequent synthesis of endocannabinoids (see [8,60,61] for a review). Different enzymes are implicated in the synthesis of AEA and 2-AG. AEA biosynthesis was originally believed to occur from enzymatic cleavage of a phospholipid precursor, N-arachidonoyl-phosphatidylethanolamine (NAPE). NAPE is synthesized by the enzymatic transfer of arachidonic acid in the sn-1 position of a phosphatidylcholine to the amide group of a phosphatidylethanolamine under the supervision of the calcium-independent N-acyl-transferase (NAT) [62]. NAPE is then hydrolyzed to AEA by a specific phospholipase D (NAPE-PLD) which has recently been cloned and molecularly characterized [8,63–65]. However, NAPE-PLD knockout mice show no deficit in AEA production, a finding which questions the role of this enzyme in anandamide biosynthesis [66]. Thus, multiple enzymatic pathways may be involved in the biosynthesis of anandamide and NAPE-PLD is unlikely to exclusively control its' biosynthesis [66,67]. 2-AG is synthesized in a two step process. First, the 2-AG precursor diacylglycerol (DAG) is formed from enzymatic cleavage of membrane phospholipid precursors by the enzyme phospholipase

C (PLC) (for review see [68,69]). DAG is subsequently hydrolyzed by a diacylglycerol lipase (DAGL) selective for the sn-1 position to generate 2-AG [8,68,70,71]. A detailed review of these processes is available [7,60,61] (see Fig. 1). Subsequent to their on-demand synthesis, endocannabinoids may activate cannabinoid receptors following their release into the extracellular space or their movement directly into the cell membrane [72]. AEA preferentially binds to CB_1 receptors *in vitro*, and exhibits low affinity for the transient receptor potential vanilloid 1 (TRPV1) [73–76]. 2-AG is known to activate both CB_1 and CB_2 receptors [50, 51]. This compound is found in the brain in concentrations 170-fold higher than those of anandamide [77]. A role for endogenous 2-AG in pain modulation has only recently been described [78,79].

In addition to activating metabotropic CB_1 receptors, AEA can also activate ionotropic TRPV1 receptors as an endovanilloid. TRPV1 receptors are expressed in nociceptive sensory neurons and can detect/respond to noxious mechanical, thermal (i.e. heat) and chemical (i.e. capsaicin) stimuli [73,75,80–83]. Capsaicin and AEA share the same binding site [84], but AEA must be found at high concentrations to activate TRPV1 receptors. Activation of TRPV1 receptors increases intracellular levels of cations such as Ca^{2+} and depolarizes the cell; these effects can also liberate calcitonin gene-related peptide (CGRP) and substance P to produce vasodilatation [73]. At high concentrations, AEA can thus exert opposing effects through activation of cannabinoid and TRPV1 receptors, respectively. A functional relationship exists between TRPV1 and CB_1 receptors in dorsal root ganglia [85], spinal cord and brain [86] and wherever these two receptors may be co-expressed in the same cell. Antagonists of TRPV1 receptors are implicated in anxiolytic effects in the brain [82]. Peripheral and central TRPV1 receptors therefore remain a viable therapeutic target.

UPTAKE OF ENDOCANNABINOIDS

Reuptake of endcannabinoids, and most notably anandamide, in the synaptic space may be facilitated by a transporter that has yet to be molecularly cloned. Pharmacological inhibitors for endocannabinoid transport have nonetheless been developed (AM404, VDM11, and others) [7,74,87,88]. AEA may accumulate in neurons and other cells by facilitated diffusion rather than employing a specific transport mechanism [89,90]. This process is saturable, temperature-dependent, does not require ATP and is driven by a transmembrane concentration gradient. The existence of a specific endocannabinoid transporter remains controversial, and new discoveries are necessary to establish beyond doubt the mechanism of endocannabinoid transport [90–93]. However, it is noteworthy that AEA uptake is selectively inhibited by a variety of pharmacological agents, consistent with the existence of a saturable component in the transport of anandamide [87,94–96] (see Fig. 1).

Since endocannabinoids are produced on demand and can be released immediately from cells, they can regulate synaptic transmission, both excitatory and inhibitory. In the CNS, endocannabinoids act as neurotransmitters. Endocannabinoids are released from depolarized postsynaptic neurons and travel to presynaptic terminals where they activate CB_1 receptors through a retrograde signaling mechanism [97–100] (see Fig. 1). The general effect is a decrease in the release of neurotransmitters such as GABA (γ-amino butyric acid) or glutamate. This retrograde signaling mechanism suggests an important modulatory role for endocannabinoids in controlling neuronal excitability and maintaining homeostasis [101].

DEGRADATION OF AEA AND 2-AG

Endocannabinoid signaling is limited by efficient degradation processes involving enzymatic hydrolysis mediated by specific intracellular enzymes. The enzymes which degrade endocannabinoids are quite well characterized and include fatty-acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) ([60,61], for a review). Inhibitors for FAAH (AM374,

URB597, URB532 and others) or MGL (URB602, OMDM169, JZL184 and Compound 11) enzymes have been described ([102]; see [7,103] for a review), although selectivity of some agents may vary considerably. FAAH hydrolyzes AEA and related compounds [103–105] whereas MGL metabolizes 2-AG [106,107]. FAAH, a membrane bound enzyme, hydrolyzes AEA in neurons and astrocytes into breakdown products arachidonic acid and ethanolamine [104,108]. The distribution of FAAH in organs of the rat has been described in detail; its activity is highest in the liver followed by the small intestine, brain, and testis (see [109] for a review). Immunohistochemical studies have mapped the distribution of FAAH in the brain. FAAH is found in the termination zone of the spinothalamic tract in the ventral posterior lateral nucleus of the thalamus [110–112]. This pathway is implicated in the transmission of nociceptive information to the brain (for review see [113]). FAAH has also been found in Lissauer's tract, in neurons of the superficial dorsal horn of the spinal cord and in dorsal root ganglion cells. Although FAAH can hydrolyze 2-AG *in vitro* [114], MGL is the predominant enzyme which controls 2-AG hydrolysis. MGL, a serine hydrolase, hydrolyzes 2-AG into breakdown products (arachidonic acid and glycerol). MGL is located on presynaptic [60,78,106] whereas FAAH is found on post-synaptic [60,103] neurons. Northern blot, immunohistochemical and *in situ* hybridization techniques have demonstrated that MGL, a presynaptic enzyme, is heterogeneously distributed in the rat brain with the highest levels observed in regions expressing CB_1 receptors, such as the cortex, thalamus, hippocampus and cerebellum [106]. MGL is localized exclusively to axon terminals, where it colocalizes with CB_1 [115]. By contrast, FAAH is a postsynaptic enzyme and may regulate AEA levels near sites of synthesis [60,103]. Although the biosynthesis and metabolism of AEA and 2-AG have been simplified here to maintain the focus of this review, it is important to mention that, in addition to hydrolysis, alternative metabolic pathways exist [67,116–118]. For example, in addition to undergoing hydrolysis, endocannabinoids undergo oxidative metabolism, through which they are transformed into other biologically active mediators [119]. Indeed, there is evidence for the metabolism of AEA and 2-AG by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 enzymes, further adding to the complexity of endocannabinoid signalling mechanisms [116,117,120,121].

ENDOCANNABINOIDS IN PAIN PATHWAYS

Cannabinoid receptors, endocannabinoids, and enzymes controling their synthesis and degradation are localized to multiple levels of the neuraxis, from the periphery to the CNS ([122]; for review see [123]). The discovery of the endocannabinoid system, the availability of antagonists for cannabinoid receptors (CB_1 and CB_2) and the generation of knockout mice lacking CB_1 and/or CB_2 and FAAH have spurred research in this growing field. Sites of action for endocannabinoids in suppressing pain were initially suggested by studies employing synthetic cannabinoids targeted at CB_1 and/or CB_2 receptors. These studies have been recently reviewed [123–126].

SUPRASPINAL LEVEL

The antinociceptive [127] and electrophysiological [128] effects produced by the systemic administration of cannabinoids are attenuated following spinal transaction. These studies implicate an important role for supraspinal sites in contributing to cannabinoid analgesic action. Direct support for supraspinal sites of cannabinoid analgesic action was derived from studies injecting synthetic cannabinoid agonists intraventricularly and locally into various brain regions (for review see [126]). Structures targeted include the periaqueductal gray (PAG) [129,130], thalamus [131], rostral ventromedial medulla (RVM) [132,133] and amygdala [134,135], among others. These studies have permitted the identification of brain regions responsible for the antinociceptive properties of cannabinoids. Activation of these sites by endocannabinoids may, therefore, produce antinociception under physiological conditions.

Neurophysiological studies by Walker's laboratory first documented that cannabinoids suppress nociceptive processing ([131,132,136]; see [126] for a review). Cannabinoids, administered systemically, suppress activity of nociceptive neurons in the spinal dorsal horn [136] and ventralposterior lateral nucleus of the thalamus, without altering the activity of purely nonnociceptive neurons [131]. Importantly, these neurophysiological effects correlate highly with the antinociceptive effects of cannabinoids, and cannot be attributed to the motor effects of the same compounds [131].

Walker's group first identified a role for endogenous AEA, released under physiological conditions, in pain modulation [137]. Electrical stimulation of the dorsolateral PAG produced antinociception in the tail-flick test and mobilized endogenous AEA, as measured by microdialysis. Importantly, this stimulation-produced analgesia was blocked by the CB_1 antagonist SR141716A, demonstrating mediation by CB_1. Intraplantar administration of formalin was also shown to increase levels of endogenous AEA in the dorsolateral PAG. Thus, noxious stimulation may produce endocannabinoid mobilization [137]. Exposure to an environmental stressor (brief continous footshock) also produces endocannabinoid-mediated stress-induced analgesia that is associated with mobilization of endogenous 2-AG and anandamide [78]. Endocannabinoid mobilization was most pronounced in dorsal midbrain fragments containing the intact PAG [78]. Endocannabinoid-mediated stress-induced analgesia is blocked by CB_1 but not by CB_2 antagonists and is insensitive to blockade by opioid (i.e. with naltexone) and TRPV1 (i.e. with capsazepine) antagonists [78,138]. Moreover, 2-AG mobilization in the PAG correlates highly with endocannabinoid-mediated stress antinociception [139]. These observations are also consistent with the ability of systemic and locally administered FAAH inhibitors (e.g. URB597, arachidonoylserotonin), endocannabinoid uptake inhibitors (e.g. VDM11) and locally administered MGL inhibitors (URB602) to enhance endocannabinoid-mediated stress antinociception through a CB_1-dependent mechanism [78,79,138]. These effects were all observed at doses that do not alter basal nociceptive thresholds. In the case of URB602, which is not appropriate for systemic use as a selective MGL inhibitor, biochemical studies confirmed that URB602, injected into the PAG, increased levels of 2-AG selectively without altering levels of AEA [78]. These studies collectively suggest a functional role for endogenous AEA and 2-AG in suppressing pain under physiological conditions.

Exogenous cannabinoids also modulate activity of ON and OFF cells in the rostral ventromedial medulla; here, inactivation of the RVM suppresses exogenous cannabinoid antinociception [133]. Pharmacological inactivation of the RVM also suppresses endocannabinoid-mediated analgesia in a rodent model of stress-induced analgesia [138]. Endocannabinoid-mediated stress-induced analgesia is also enhanced in a CB_1-dependent manner by intra-RVM administration of a FAAH inhibitor, administered at doses that do not alter the basal nociceptive threshold [138]. These studies support a role for endogenous AEA in the RVM in endocannabinoid-mediated analgesia, although a role for 2-AG has not been assessed.

Endocannabinoid levels are altered following nerve injury in specific brain regions implicated in cannabinoid antinociceptive mechanisms. For example, injury of the sciatic nerve increases AEA and 2-AG levels in the PAG and RVM [140], structures implicated in both the descending modulation and the descending facilitation of pain (see [113] for review). AEA levels were also increased in the dorsal raphe following chronic constriction injury (CCI) [140,141].

Systemic administration of inhibitors of endocannabinoid uptake (VDM-11, OMDM-2, UCM-707 and LY2318912) increases AEA and/or 2-AG levels in brain [93,142]. Interestingly, FAAH inhibition by N-arachidonoyl-serotonin (AA-5-HT) was shown to increase brain levels

of AEA and 2-AG [142]. These studies suggest that inhibitors of endocannabinoid uptake and deactivation show therapeutic potential for increasing endocannabinoid levels.

SPINAL LEVEL

Intrathecal administration of cannabinoids produces antinociception [143–145], and suppresses nociceptive neuronal activity [146]. These studies initially documented the existence of spinal sites of cannabinoid antinociceptive actions. Indeed, behavioral [143,145], electrophysiological [146–148] and neurochemical [128,149] studies have demonstrated that cannabinoids act at the spinal level to modulate pain. Mixed cannabinoid agonists such as levonantradol [145], WIN55,212-2 [150] and CP,55,940 [151], at the spinal level, produce CB_1-mediated antinociceptive effects. Moreover, cannabinoids suppress C-fiber-evoked responses of dorsal horn neurons recorded in normal, inflamed and nerve injured rats [152–155]. Furthermore, these data are consistent with the ability of cannabinoids to suppress Fos protein expression, a neurochemical marker of sustained neuronal activation, in different animal models of persistent pain through CB_1 and CB_2-specific mechanisms [128,156–159]. Cannabinoid receptors have been demonstrated on primary afferents neurons at pre- and post-synaptic sites in the spinal cord using receptor binding and quantitative autoradiography [160,161]. In the dorsal horn of the spinal cord, CB_1 receptors have been found on interneurons [29] and on astrocytes [162].

Upregulation of cannabinoid receptors is also observed in the spinal cord following nerve injury [150,163], suggesting that both endocannabinoids and their receptors are regulated under conditions of injury. Exposure to an acute environmental stressor increases 2-AG, but not anandamide, accumulation in the lumbar spinal cord; 2-AG accumulation in the spinal cord correlates highly with the appearance of stress antinociception [79]. Intrathecal administration of inhibitors of both FAAH (URB597/AA5-HT) and MGL-preferring (URB602) also enhance endocannabinoid-mediated stress-induced analgesia through a CB_1-dependent mechanism.

AEA and 2-AG are also increased in the spinal cord following induction of a neuropathic pain state produced by CCI of the sciatic nerve [140]. The endocannabinoid system is similarly modulated in response to a spinal cord contusion in rats [164]. The early stages are marked by increases in AEA levels, upregulation of the synthetic enzyme NAPE-PLD, and downregulation of the degradative enzyme FAAH. The delayed stages are marked by increases in 2-AG, a marked upregulation of the 2-AG synthesizing enzyme DAGL-α (i.e. in neurons, astrocytes and immune infiltrates), and a moderate increase in levels of the degradative enzyme MGL [164]. In this study, CB_1 receptors were expressed in neurons, oligodendrocytes, and astrocytes, whereas CB_2 receptors were strongly upregulated after the lesion and expressed mainly in immune infiltrates and astrocytes [164]. These studies highlight the importance of the endocannabinoid system as a potential therapeutic target for treatment of both spinal cord injury and neuropathic pain.

PERIPHERAL LEVEL

Peripheral antinociceptive actions of cannabinoids have been demonstrated in numerous animal pain models (for review see [123–125]). Harnessing these mechanisms shows considerable promise for separating the therapeutic effects of cannabinoids from unwanted CNS side-effects. Cannabinoid receptors are synthesized in dorsal root ganglion (DRG) cells, which are the source of primary afferent input to the spinal cord [30,31,85,165–167]. These afferent nerve fibers transmit information about sensory stimulation to the spinal cord, thereby enabling communication between the periphery and specific areas of the CNS that contribute to pain perception [168,169]. Following the induction of neuropathy (by spinal nerve ligation), cannabinoid receptors and their endogenous ligands (AEA and 2-AG) are increased in the DRG on the ipsilateral side of the injury [170]. Cannabinoid CB_1 [30,31,85,162] and CB_2 receptors

[165,167] are also found in the DRG. DRG cells synthesize cannabinoid receptors, and transport them to peripheral terminals of primary afferents [30,31]. Multiple approaches support the presence of cannabinoid receptors on primary afferent neurons [85,166,171]. CB_1 and CB_2 receptors are found in large myelinated and small unmyelinated human cutaneous nerve fibers [166]. Both cannabinoid receptor subtypes have also been found in different layers of the skin, and in some adnexal structures (sweat glands, sebaceous cells and others) which may contribute to peripheral antinociceptive actions [166,172–175]. Endocannabinoid levels and FAAH activity have also been measured in rodent paw skin [176–179]. AEA is observed in paw tissue [177–178] whereas a decrease in FAAH activity is observed in the inflamed paw following carrageenan-induced inflammation [179]. In the formalin model, 2-AG hydrolysis inhibitor, OMDM169, increased levels of 2-AG, but not AEA, in the ipsilateral paw [180]. However, Beaulieu and collaborators did not find an increase in AEA and 2-AG levels in the formalin test, measured 2 h after formalin injection when pain behavior has subsided [176]. In a model of bone cancer pain, intraplantar administration of exogenous AEA or the FAAH inhibitor URB597 increased the local level of AEA [181]. These studies suggest that manipulation of peripheral endocannabinoids may be promising strategy for the management of pain.

MODULATION OF THE ENDOCANNABINOID SYSTEM IN ANIMAL MODELS

Studies evaluating the presence of hypersensitivity to pain (hyperalgesia) following pharmacological blockade of CB_1 receptors provided early physiological support for the hypothesis that endocannabinoids suppress pain. Hyperalgesia has been observed in the hotplate test following intrathecal administration of the CB_1 antagonist/inverse agonist SR141716A, and these effects are mimicked by CB_1 antisense knockdown at the spinal level [182,183]. Pharmacological blockade of CB_1 receptors with SR141716A has also been reported to produce hyperalgesia in the formalin test [177,184]. However, these findings have not been observed by all investigators [176], suggesting that differences in the level of endogenous analgesic tone may contribute to differences between the studies (see [126] for review). Moreover, the inverse agonist properties of SR141716A complicate interpretation of studies attempting to unmask tonic endocannabinoid activity using competitive antagonists.

Therefore, documentation of intrinsic effects of endocannabinoids released under physiological conditions is critical for understanding the functional roles of endocannabinoids in nociceptive processing. As described above, studies employing stimulation-produced analgesia and stress-induced analgesia provide direct support for the hypothesis that endogenous AEA and 2-AG suppress pain through a CB_1-dependent mechanism. In these studies, the tail-flick test was used to quantify the impact of electrical brain stimulation or exposure to footshock stress on antinociception. Thus, it is important to emphasize that treatment with CB_1 antagonists [79,128,137,138,160] or modulators of endocannabinoid transport or deactivation [79,128,138,160] lacked intrinsic effects in the tail-flick test in the absence of a stimulus to trigger endocannabinoid mobilization (i.e. brain stimulation or footshock exposure). Thus, it is important to emphasize that tail-flick stimulation is not the trigger for endocannabinoid mobilization in these studies, and antagonists do not alter basal nociceptive thresholds under testing conditions. A role for CB_2 was not evaluated in studies of endocannabinoid-mediated stimulation-produced analgesia, presumably due to the lack of availability of a CB_2 antagonist at the time the work was conducted [137]. Stress-induced analgesia is also CB_1-mediated; it is blocked by multiple CB_1 antagonists, involves the mobilization of endocannabinoids at supraspinal (2-AG and AEA; [78]) and spinal (2-AG alone; [79]) levels and is enhanced by inhibitors of endocannabinoid deactivation (URB597, AA-5-HT, URB602) or transport (VDM11). The failure of a CB_2 antagonist to block endocannabinoid-mediated stress-induced analgesia in these studies [78] may reflect the absence of a CB_2-mediated component in endocannabinoid-mediated stress analgesia or,

alternately, the failure of the spinally-mediated tail-flick test to detect a CB_2-mediated component of endocannabinoid analgesia. The existence of a cross-tolerance and cross-sensitization between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia suggests that these phenomena are linked by a common mechanism [185]. The development of drugs that inhibit the enzymatic degradation of endocannabinoids (i.e. through inhibition of FAAH or MGL) or their transport has improved our understanding of the functional roles of the endocannabinoid system in modulating pain under physiological conditions.

Effects of exogenous administration of endocannabinoids (focusing on AEA and 2-AG) and their modulation in models of acute, inflammatory and neuropathic pain models are reviewed below. However, one limitation of studies employing exogenous endocannabinoids is that they do not demonstrate that the endogenous ligands play similar roles under physiological conditions.

ACUTE NOCICEPTION

Exogenous administration of endocannabinoids or their modulation via inhibition of endocannabinoid deactivation or uptake can produce antinociception in acute pain models (see Table 1 and Table 2). The magnitude of the observed antinociceptive effect may differ depending upon the assay, the endocannabinoid used and/or the mechanism employed to alter endocannabinoid levels. The *tail flick test* examines the latency for a rodent to “flick” its tail away from a radiant heat source [186], or to remove the tail following immersion in hot water (see Table 1). In this test, the endocannabinoid uptake inhibitors (VDM-11 and UCM707) produce CB_1-mediated antinociception [187] under conditions in which the endocannabinoid system is activated [78]. Exogenous administration of AEA produces antinociception [188–191], although few studies have evaluated whether this effect is mediated by cannabinoid receptors. Several groups have evaluated a CB_1 component in exogenous AEA antinociception [192–194], but other studies have suggested that anandamide produces antinociception through a CB_1-independent mechanism [188,191]. All these studies assessed pharmacological specificity using the CB_1 antagonist/inverse agonist SR141716A antagonist. Thus, it is important to emphasize that SR141716A acts as an inverse agonist at CB_1 receptors and can activate both CB_2 and vanilloid TRPV1 receptors, albeit with low affinity (for review see [195]). Moroever, a role for CB_2 receptors cannot be discounted from contributing to the antinociceptive effects of exogenous administration of AEA, because mediation by CB_2 receptors was not assessed in these studies. MGL (URB602) and FAAH (AA-5-HT, PMSF, PTK, URB597) inhibitors with varying degrees of selectivity also produce antinociceptive effects in the tail flick test [189,196,197], and specifically under conditions in which the endocannabinoid system is activated and basal nociceptive thresholds are not altered by the same treatments ([79,138] for FAAH inhibitors only; [78] for MGL and FAAH inhibitors). In these studies, cannabinoid receptor antagonists directed at CB_1 (AA-5-HT, PTK, URB597 and URB602 [7879,138]) or at CB_1/CB_2 (URB597 [197]) were used to identify the receptor mechanism underlying these effects. Indeed, studies employing FAAH knockout mice also corroborate the previous results; a CB_1-mediated component is observed in both the tail immersion and hot plate tests under conditions in which both CB_1 and CB_2 antagonists were evaluated [198]. The combination of exogenous AEA with FAAH (ibuprofen, indomethacin, PMSF, URB597) inhibitors also produces antinociception [189,191,196] that is mediated by CB_1 receptors [189,191].

The *hot plate test* involves individually placing rodents on a metal surface typically maintained at 52°C (Range: 52–58°C for these studies) and measures the latency for the rats to exhibit the first sign of pain (i.e. licking the hind paws or jumping) [199] (see Table 2). In this procedure, inhibitors of endocannabinoid uptake (UCM707, OMDM-2, VDM-11) produce

antinociception, although mediation by cannabinoid receptors has not been assessed using competitive antagonists [200,201]. Moreover, exogenous administration of AEA produces an antinociceptive effect in the hotplate test [192,202,203] that seems to be CB_1-mediated [192] (see Table 2). Consistent with this observation, FAAH inhibitors (URB597 and URB532) produce CB_1-mediated antinociception [204]. Endocannabinoid uptake inhibitors (UCM707 and OMDM-1) also potentiate the antinociceptive effect of exogenous anandamide at a dose that did not produce an effect when given alone [200,201]. These observations are consistent with the CB_1-mediated enhancement of endocannabinoid-mediated stress analgesia produced by the uptake inhibitor VDM11 in the tail-flick test [78].

The *plantar test* measures the latency for animals to remove their paws from a radiant heat source that is focused onto the plantar surface of the paw through the floor of a glass platform [205]. In this test, the FAAH inhibitor Compound 17 dose-dependently potentiates the effects of exogenous AEA in the plantar test [206]. Finally, exogenous administration of AEA also produces CB_1-mediated antinociception in the *paw pressure test* [207], assessed using the method of Randall and Selitto [208] (see Table 2). A role for cannabinoid CB_2 receptors in antinociception in otherwise naive animals has been studied in an attempt to optimize the therapeutic potential of cannabinoid analgesic systems. CB_2 agonists show therapeutic potential because they are devoid of the unwanted central side-effects attributed to activation of CB_1 receptors ([124] for a review). However, previous studies assessing responsiveness to acute nociceptive stimulation have either not typically examined the role of CB_2 in mediating effects linked to endocannabinoids (AEA and 2-AG), or have not supported the involvement of CB_2 mechanisms in endogenous analgesia [78]. It is therefore acknowledged that only certain assays (e.g. the plantar test) are likely to be sensitive to detection of CB_2-mediated antinociceptive effects in the absence of inflammation or injury (for review see [124]). Thus, animal models of persistent pain are likely to be differentially sensitive to CB_2-mediated components of cannabinoid antinociception. Thus, manipulation of endocannabinoid accumulation through inhibition of metabolism or reuptake mechanisms may be employed to elucidate a role for cannabinoid CB_2 receptors under conditions of inflammation or injury.

PERSISTENT INFLAMMATORY NOCICEPTION

Cannabinoids produce antinociception in tissue injury models of persistent pain. Indeed, behavioural, electrophysiological and neurochemical studies all support a role for cannabinoid CB_1 and CB_2 receptors in modulating inflammatory nociception (for review see [126]). Effects of exogenous administration of endocannabinoids and/or their modulators (i.e. inhibitors of endocannabinoid uptake or hydrolysis) in different inflammatory pain models (formalin, carrageenan, capsaicin, complete Freund's adjuvant,) is discussed separately because the mechanisms underlying the development and maintenance of distinct inflammatory pain states differs (see Table 3 and Table 4).

The *formalin test* is a well-established model of persistent pain characterized by a transient, biphasic pattern of pain behaviour [209]. The early phase is characterized by acute activation of C and Aδ fibers. The late phase involves an inflammatory reaction in peripheral tissue [210], the development of central nervous system sensitization [211,212] and additionally involves activation of primary afferent nociceptors [213]. In the formalin test, endocannabinoid uptake inhibitors (AM404, UCM707, LY2318912, LY2183240, OMDM132) produce antinociception [93,214,215] (see Table 3). These antinociceptive effects are mediated either exclusively by CB_1 receptors [214,215] or by both CB_1 and CB_2 receptors [215]. Exogenous AEA produces CB_1-dependent antinociception in the formalin test [177,216] whereas exogenous 2-AG predominantly produces CB_2-dependent antinociception [217]. The formalin test has also been used to assess antinociceptive effects produced by FAAH inhibitors (MAFP, Flurbiprofen, Ibuprofen, Compound 17, propofol, AA-5-HT, OMDM106, LY2183240 and

others) [178,206,215,216,218–220]. Thus, it is noteworthy that the mechanism of action varies with the compound employed. For example, AA-5-HT [219,220] and LY2183240 produce CB_1-mediated antinociception [215] whereas propofol, a widely used general anesthetic, mediates its antinociceptive effects through activation of CB_1 and CB_2 receptors [221] (see Table 3). FAAH knockout mice also exhibit CB_1-mediated hypoalgesia in both phases of the formalin test [198]. However, the nonsteroidal anti-inflammatory drug ibuprofen produces antinociception in the formalin test that is not related to cannabinoid or TRPV1 receptors [216]. Both CB_1 and CB_2 receptors are implicated in the antinociceptive effects of MGL inhibitors (OMDM169 and URB602) in this test [180,217]. Furthermore, the combination of AEA with nonselective FAAH inhibitors (ibuprofen or rofeocoxib) produces an antinociceptive effect [178,216] that is CB_1-mediated [216], whereas the combination of 2-AG with URB602 produces antinociception whose mechanism of action remains to be determined [217].

The *carrageenan model* involves intraplantar injection of the inflammatory agent, carrageenan, which produces paw swelling (edema) and hypersensitivity to mechanical or thermal stimulation [205]. Carrageenan also induces the expression of Fos, a nonspecific marker of neuronal activation, in the lumbar spinal cord [222]. In this model, exogenous administration of anandamide produces antinociception [183,223,224] which is likely to be CB_1-mediated [183] (see Table 4). FAAH inhibitors (URB597 and JNJ-1661010) also produce antinociception in this model ([179,225,226] using URB597; [227] using JNJ-1661010). However, this antinociceptive effect is likely to be independent of CB_1 receptor activation because a CB_1 antagonist failed to reverse the observed antinociceptive effects [179,226]. A role for both CB_2 receptors [179] and peroxisome proliferator-activated receptor-α (PPAR-α) receptors has been implicated in the antinociceptive effects of URB597 in this model [226]. A role for CB_2 but not CB_1 receptors in thermal anti-hyperalgesic effects exhibited by FAAH knockout mice has also been demonstrated; however, neither CB_1 nor CB_2 receptors are implicated in the anti-edemic effects of $FAAH^{-/-}$ mice [198]. Although highly specific MGL inhibitors have only recently been described, MGL-selective inhibitors (URB602 and compound 21) nonetheless exhibit antinociception in this model [228,229; respectively), an effect which involves CB_2 receptors [228]. However, caution must be exerted in interpreting effects of URB602, which also inhibits FAAH, and thus, is unlikely to act as a selective MGL inhibitor following systemic administration.

Capsaicin, the pungent ingredient in hot chili peppers, produces hypersensitivity to mechanical and thermal stimulation as well as spontaneous pain following intradermal administration [230]. Hyperalgesia evoked in *capsaicin model* refers to an increase in pain behavior evoked by suprathreshold stimuli and/or a lowered threshold for pain [230,231]. Only one study has assessed antinociceptive effects following exogenous administration of AEA [202] without investigating the cannabinoid (CB_1 and/or CB_2) receptor mechanism of action (see Table 4). *Complete Freund's adjuvant* (CFA), administered in the plantar hindpaw surface, produces peripheral edema as well as hypersensitivity to mechanical and thermal stimulation in rodents [232–234]. The inflammation appears approximately two hours following injection of complete Freund's adjuvant, produces its maximal effect after six to eight hours and can persist for weeks following injection [233,235]. Exogenous administration of AEA produces antinociception in the CFA model, but this effect does not involve CB_1 receptors [207]. A CB_2 mechanism of action was not investigated in this study, likely due to the lack of available CB_2-selective antagonists at the time of testing. In this model, the antinociceptive effect of the FAAH inhibitor URB597 is mediated by both CB_1 and CB_2 receptors [236]. Furthermore, AM404, an inhibitor of endocannabinoid uptake, produces CB_1-mediated antinociception in the CFA model [214,237] (see Table 4). These observations are consistent with the ability of exogenous anandamide to produce antinociception in other inflammatory pain models (acid

acetic writhing test, kaolin writhing test, and other models) through a CB_1-dependent mechanism [202,238] (see Table 4).

NEUROPATHIC NOCICEPTION

Animal models of neuropathic pain have been developed to mimic symptoms associated with nerve injury observed clinically. Neuropathic pain can be induced by traumatic nerve injury [239–241], toxic insults and metabolic challenges. Pharmacotherapies used to treat neuropathic pain produce inadequate pain relief and/or unwanted side-effects (for review see [124,242]), which reinforce the importance of identifying and validating novel therapeutic approaches which suppress neuropathic pain, including those targeting the endocannabinoid system (see Table 5). The *chronic constriction injury model* is a widely used animal model of neuropathic pain that is produced by loosely placing three constrictive ligatures around the common sciatic nerve [239]. In this model, inhibition of endocannabinoid uptake with AM404 produces antinociceptive effects which are mediated by CB_1 [141,214,243,244] and partially by CB_2 receptors [243]. However, discrepancies between studies are also apparent [214,244] (see Table 5). The endocannabinoid uptake inhibitor VDM11 also produces antinociceptive effects, but involvement of cannabinoid receptors in these effects has not been evaluated [243]. FAAH inhibitors (URB597, AA-5-HT, OL-135) also produce antinociception in the CCI model [219,245]. The FAAH inhibitor URB597 produces antihyperalgesic effects in this model that are CB_1-mediated and partially CB_2-mediated. By contrast, another FAAH inhibitor (AA-5-HT) has been shown to produce antihyperalgesia that is mediated exclusively by CB_1 receptors. No genotype differences in pain behavior were observed between $FAAH^{-/-}$ and wildtype mice subjected to a chronic constriction injury [198]. However, nerve injury may promote adaptive changes in these animals because CCI was found to obliterate the phenotypic hypoalgesia displayed by $FAAH^{-/-}$ mice in the tail immersion and hot plate tests [198].

Pharmacological modulation of endocannabinoid levels also suppresses neuropathic pain behavior in other models of surgically-induced traumatic nerve injury. For example, AM404 produces CB_1-dependent antinociception [237] in a model of unilateral hind limb neuropathy induced by *partial sciatic nerve ligation* (PSNL) [240]. Exogenous administration of anandamide similarly produces CB_1-dependent antinociceptive effects [246,247] whereas the antinociceptive effects of 2-AG, administered via the same route, are CB_1/CB_2 mediated [248] (see Table 5). FAAH inhibitors (URB597, Ibuprofen, Rofecoxib) are also antinociceptive in this model [246,248]. URB597 produces antinociception through a local peripheral mechanism that is mediated by CB_1/CB_2 cannabinoid receptors [248]. However, systemic administration of the same compound does not reliably produce antinociception [236]. Moreover, antinociception produced by local injection of ibuprofen and rofecoxib in the paw does not involve CB_1 or CB_2 cannabinoid receptors [246]. Local administration of URB602 also produces a CB_1/CB_2 antinociception in this model [248]. The combination of FAAH or MGL inhibitors with the exogenous administration of endocannabinoids (AEA or 2-AG) also enhances the antinociceptive effects of the putative endocannabinoid [246,248], but the mechanism of action remains to be determined. The combination of AEA with either ibuprofen or rofecoxib produces antinociception that is mediated exclusively by CB_1 receptors, although the mechanism of action for these other combinations remains to be investigated [246].

Effects of modulation of the endocannabinoid system on neuropathic pain behavior have recently been evaluated using a *spinal nerve ligation model* (SNL). Neuropathic pain was induced by ligating the L5 and L6 spinal nerves according to the procedures described by Kim and Chung [241]. In this model, FAAH inhibitors (URB597, Compound 17, JNJ-1661010 and Compound 34) have been studied exclusively [206,227,249,250] (see Table 5). The antinociceptive effects produced by these agents may involve non-cannabinoid receptor mechanisms (e.g. PPAR-α). However, antinociception produced by URB597 has been shown

to involve CB_1 receptors [249]. Thus, antinociception produced by FAAH/MGL/endocannabinoid uptake inhibitors are influenced by the compound employed, the animal model used and potentially the level of endocannabinoid tone produced by the injury. Thus, systemic administration of URB597 produces CB_1-mediated enhancements of stress antinociception at doses that do not alter basal nociceptive thresholds in the tail flick test (Table 1). However, systematically administered URB597 produces CB_2-mediated antinociception in the carrageenan model and CB_1/CB_2-mediated antinociception in complete Freund's adjuvant, partial sciatic nerve ligation (local injection) and chronic constriction injury models.

Interpretation of effects of URB602 is more complicated as this compound is not MGL-selective, and can inhibit FAAH; URB602 produces CB_2-mediated antinociception in the carrageenan model (systemic injection) and CB_1/CB_2-mediated antinociception in the formalin test (i.e. following local injection) and partial sciatic nerve ligation (i.e. following local injection) models (see Table 3–Table 5). Thus, effects of URB602 are only likely to be mediated by MGL under conditions in which it is documented that local administration of URB602 increases 2-AG accumulation without altering levels of AEA [78]. Systemic administration of AM404 produces CB_1-mediated antihyperalgesic effects in inflammatory pain models such as complete Freund's adjuvant and formalin models but involves CB_1/CB_2 receptors in the CCI model. Moreover, local exogenous administrations of 2-AG produce CB_2-mediated antinociception in the formalin test and CB_1/CB_2-mediated antinociception in the partial sciatic nerve ligation model. However, local administration of AEA produces CB_1-mediated antinociception in both of these models (see Table 3–Table 5). A local route of agonist administration may unmask CB_2-mediated components in the antinociceptive effects produced by pharmacological inhibitors of endocannabinoid uptake and degradation. However, URB597 produces antinociceptive effects with largely consistent pharamacological specificity following either systemic or local routes of administration. It is also important to emphasize that inhibitors of FAAH elevate levels of fatty-acid amides that do not bind to cannabinoid receptors (e.g. palmitoylethanolamine) and have targets (e.g. PPAR-α) that are distinct from CB_1 and CB_2 receptors. Thus, the contribution of non-cannabinoid receptor mechanisms of action in the *in vivo* pharmacological effects of FAAH and MGL inhibitors must also be considered.

LIMITATIONS

This review focuses on understanding the functional consequences of increasing endocannabinoid accumulation through blockade of endocannabinoid deactivation or transport, with the caveat that many of these agents employed (e.g. FAAH or MGL inhibitors) are not selective for the endocannabinoid system. Moreover, increasing specific endocannabinoids (e.g. anandamide) or fatty-acid amides (e.g. palmiotylethanolamine) can activate other non-cannabinoid receptors (e.g. TRPV1 or PPAR-α, respectively). Entourage effects may also be produced by manipulations that elevate levels of endogenous lipid mediators that do not bind to cannabinoid receptors but, nonetheless, compete for the same enzymes for hydrolysis [251]. Thus, not all effects of these modulators can be attributed to actions at cannabinoid receptors, and assessment of pharmacological specificity is critical for interpretation of *in vivo* actions of any compound. Palmitoylethanolamide (PEA), an endogenous fatty-acid ethanolamide, is an agonist at PPAR-α receptors, but does not bind to cannabinoid receptors [252,253]. However, effects of this compound can nonetheless be blocked by the CB_2 antagonist SR144528 [177]. Inhibition of FAAH by URB597 can also produce antinociceptive effects in inflammatory pain models that are mediated by the activation of PPAR-α receptors [225,226,254]. Synergistic interactions between anandamide and GW7647 (PPAR-α agonist) have been demonstrated in the formalin test [255]. Thus, modulation of the endocannabinoid system by FAAH/MGL/uptake inhibitors and their possible interaction with non-cannabinoid receptors requires further investigation. Even

though increases in endocannabinoid accumulation are produced by inhibition of the degradative enzymes described in this review, differences in selectivity or potency and heretofore uncharacterized off-target effects may complicate interpretation of results. Therefore, the reader should be aware of these limitations when interpreting the results of any specific study.

CONCLUSION

Endocannabinoids modulate pain under physiological conditions. Pharmacological approaches that enhance levels of endocannabinoids by inhibiting enzymes controling endocannabinoid deactivation or by blocking their reuptake consequently exhibit therapeutic potential. It is clear that the endocannabinoid system is regulated following conditions of injury. Therefore, more work is necessary to better understand the broad consequences of pharmcological approaches that modulate endocannabinoid levels. Inhibition of endocannabinoids deactivation is likely to show a more beneficial and circumscribed spectrum of biological effects compared to direct activation of CB_1 receptors; effects of these inhibitors are limited to sites where endocannabinoids are mobilized under physiological conditions in a stimulation-contingent fashion. Limitations to therapeutic approaches which modulate the endocannabinoid system (e.g. in immunosuppressive diseases) should also be considered when assessing the therapeutic potential of any approach. The impact of long term treatment should be assessed. Multimodal approaches combining modulation of endocannabinoid with other conventional analgesics (e.g. NSAIDs) should also be evaluated for their therapeutic potential. Adjunctive approaches show strong promise for improving the efficacy of existing pharmacotherapies for pain and limiting unwanted side-effect profiles.

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ABBREVIATIONS

2-AG	2-arachidonoylglycerol
AA	arachidonic acid
AA-5-HT	N-arachidonoyl serotonine
AEA	anandamide
Ca^{2+}	calcium
CB_1	cannabinoid receptor 1
CB_2	cannabinoid receptor 2
CCI	chronic constriction injury
CGRP	calcitonin gene-related peptide
CNS	central nervous system
COX	cyclooxygenase
Δ^9-THC	delta 9-tetrahydrocannabinol
DAG	diacylglycerol
DAGL	diacylglycerol lipase
DR	dorsal raphe

DRG	dorsal root ganglion
ET	endocannabinoid membrane transporter
FAAH	fatty acid amide hydrolase
GABA	γ-amino butyric acid
LOX	lipoxygenase
MAFP	methyl arachidonyl fluorophosphonate
MAPK	mitogen-activated protein kinase
MGL	monoacylglycerol lipase
NADA	N-arachidonoyldopamine
NAPE	N-arachidonoyl-phosphatidylethanolamine
NAT	N-acyl transferase
NT	not tested
PAG	periaqueductal gray
PG	prostaglandins
PLC	phospholipase C
PLD	phospholipase D
PMSF	phenylmethylsulfonyl fluoride
PSNL	partial sciatic nerve ligation
PTK	palmitoyltrifluoromethylketone
RVM	rostral ventromedial medulla
SIA	stress-induced analgesia
SNL	spinal nerve ligation
TRPV1	transient receptor potential vanilloid 1

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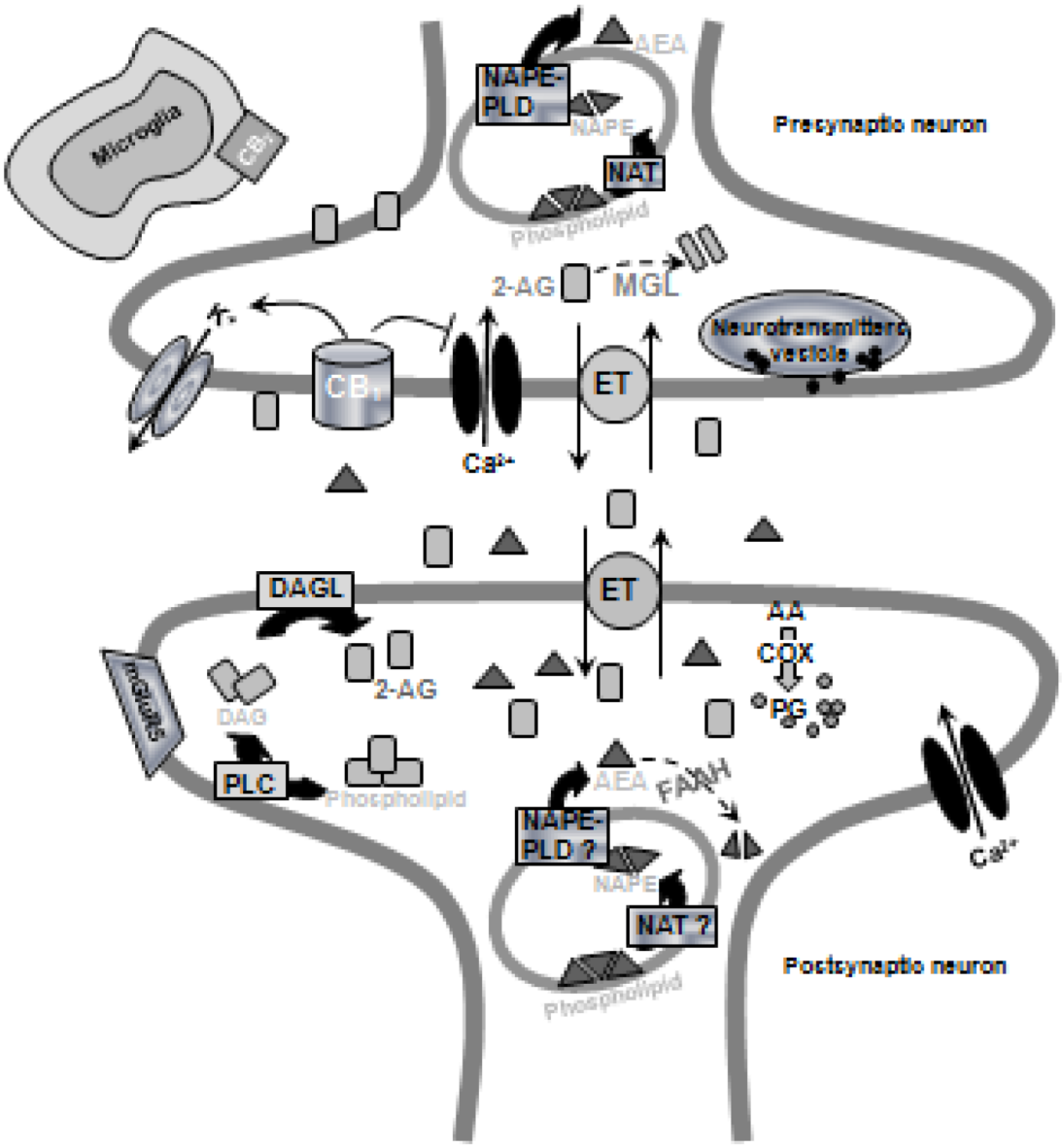

Figure 1.
Formation and inactivation of anandamide and 2-arachidonoylglycerol

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Table 1

Antinociceptive effects of modulators of the endocannabinoid system in the tail flick model

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
Tail flick	Endocannabinoid uptake inhibitors	VDM-11	Yes during SIA	Yes	NT	78
		UCM707	Yes	Yes	NT	187
	Exogenous endocannabinoids	AEA	Yes	NT	NT	190
			Yes	NT	NT	256
			Yes	NT	NT	203
			Yes	No	NT	188
			Yes	Yes	NT	194
			Yes trend	Yes	NT	192
			Yes	Yes	NT	193
			Yes	NT	NT	257
			Yes	No	NT	191
			Yes	NT	NT	189
			Yes	NT	NT	224
	FAAH inhibitors	PMSF	Yes	NT	NT	196
		PTK	Yes during SIA	Yes	NT	138
		AA-5-HT	Yes during SIA	Yes	NT	79, 138
		URB597	Yes during SIA	Yes	NT	78
			Yes during SIA	Yes	NT	79
			Yes	NT	NT	189
			Yes	Yes	No	197

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
	MGL inhibitors	URB602	Yes during SIA	Yes	NT	78
			Yes during SIA	Yes	NT	79
	Exogenous Endocannabinoids+ FAAH inhibitors	AEA + PMSF	Yes	NT	NT	196
			Yes	Yes	NT	191
		AEA + Ibuprofen	Yes	NT	NT	191
		AEA + Indomethacin	Yes	NT	NT	191
		AEA + URB597	Yes	Yes	No	189

Table 2

Antinociceptive effects of modulators of the endocannabinoid system in acute pain models

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
Hot plate	Endocannabinoid uptake inhibitors	UCM707	Yes	NT	NT	200
		OMDM-2	Yes	NT	NT	201
		VDM-11	Yes	NT	NT	201
		O-2093	No	NT	NT	258
	Exogenous endocannabinoids	AEA	Yes	NT	NT	203
			Yes	NT	NT	87
			Yes trend	Yes	NT	192
			Yes	NT	NT	202
	FAAH inhibitors	URB597	Yes	Yes	NT	204
		URB532	Yes	Yes	NT	204
	Endocannabinoid uptake inhibitors + Exogenous endocannabinoids	AM404 + AEA	Yes	NT	NT	87
		UCM707 + AEA	Yes	NT	NT	200
		OMDM-1 + AEA	Yes	NT	NT	201
Plantar	Exogenous Endocannabinoids+ FAAH inhibitors	AEA + Compound 17	Yes	NT	NT	206
Paw Pressure Test	Exogenous Endocannabinoids	AEA	Yes	Yes	NT	207

Table 3

Antinociceptive effects of modulators of the endocannabinoid system in the formalin model of inflammation

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
Formalin Test	Endocannabinoid uptake inhibitors	LY2318912	Yes	NT	NT	93
		UCM707	Yes	NT	NT	214
		AM404	Yes	Yes	No	214
		LY2183240	Yes	Yes	No	215
		OMDM132	Yes	Yes	Yes	215
	Exogenous Endocannabinoids		Yes	NT	NT	259
			Yes	Yes	No	177
		AEA	Yes	Yes	No	216
			Yes	NT	NT	255
		2-AG	Yes	No	Yes	217
	FAAH inhibitors	MAFP	Yes	Yes	NT	218
		Flurbiprofen	Yes	Yes	NT	218
		Ibuprofen	Yes	No	No	216
		Rofecoxib	Yes	NT	NT	178
		Propofol	Yes	Yes	Yes	221
		AA-5-HT	Yes	Yes	No	219
		AA-5-HT	Yes	Yes	No	220
		OMDM106	Yes	Yes	NT	220
		Compound 17	Yes	Yes	NT	206
		OMDM119	Yes	No	NT	215
		OMDM122	Yes	No	NT	215

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
		LY2183240	Yes	Yes	No	215
	MGL inhibitors	URB602	Yes	Yes	Yes	217
		OMDM169	Yes	Yes	Yes	180
	Exogenous Endocannabinoids + FAAH inhibitors	AEA + Ibuprofen	Yes	Yes	No	216
		AEA + Rofecoxib	Yes	NT	NT	178
	Exogenous Endocannabinoids+ MGL inhibitors	2-AG + URB602	Yes	NT	NT	217

Table 4

Antinociceptive effects of modulators of the endocannabinoid system in inflammatory pain models

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
Carrageenan	Exogenous Endocannabinoids	AEA	Yes	NT	NT	223
			Yes	Yes	NT	183
			Yes	NT	NT	224
	FAAH inhibitors	URB597	Yes	No	Yes	179
			Yes	NT	NT	225
			Yes	No	NT	226
		JNJ-1661010	Yes	NT	NT	227
	MGL inhibitors	URB602	Yes	No	Yes	228
		Compound 21	Yes	NT	NT	229
Capsaicin	Exogenous Endocannabinoids	AEA	Yes	NT	NT	202
Complete Freund's Adjuvant	Endocannabinoid uptake inhibitors	AM404	Yes	Yes	No	214
			Yes trend	NT	NT	237
	Exogenous Endocannabinoids	AEA	Yes	No	NT	207
	FAAH inhibitors	URB597	Yes	Yes	Yes	236
Acetic Acid Writhing Test	Exogenous Endocannabinoids	AEA	Yes	Yes	No	202

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
	MGL inhibitors	Compound 21	Yes	NT	NT	229
Kaolin Writhing Test	Exogenous Endocannabinoids	AEA	Yes	Yes	No	202
NGF inflammatory hyperalgesia	Exogenous Endocannabinoids	AEA	Yes	Yes	No	238
p-phenyl-quinone stretch test	Exogenous Endocannabinoids	AEA	Yes	NT	NT	203
Turpentine bladder inflammation	Exogenous Endocannabinoids	AEA	Yes	NT	NT	259

Table 5

Antinociceptive effects of modulators of the endocannabinoid system in neuropathic pain models

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
CCI	Endocannabinoids uptake inhibitors	AM404	Yes	Yes	Yes	243
			Yes	Yes	No	214,244
			Yes	Yes	NT	141
		VDM11	Yes	NT	NT	243
	FAAH inhibitors	URB597	Yes	NT	NT	219
			Yes	Yes	Yes	245
		AA-5-HT	Yes	Yes	No	219
		OL-135	Yes	NT	NT	219
PSNL	Endocannabinoids uptake inhibitors	AM404	Yes	Yes	NT	237
	Exogenous Endocannabinoids	AEA	Yes	Yes	No	247
			Yes	Yes	No	246
		2-AG	Yes	Yes	Yes	248
	FAAH inhibitors	URB 597	No	NT	NT	236
			Yes	Yes	Yes	248
		Ibuprofen	Yes	No	No	246
		Rofecoxib	Yes	No	No	246
	MGL inhibitors	URB602	Yes	Yes	Yes	248

Pain Model	Compounds		Anti-nociception	Mediated by:		Studies
				CB_1	CB_2	References
	Exogenous Endocannabinoids+ FAAH inhibitors	AEA + Ibuprofen	Yes	Yes	No	246
		AEA + Rofecoxib	Yes	Yes	No	246
		2-AG + URB597	Yes	NT	NT	248
	Exogenous Endocannabinoids+ MGL inhibitors	2-AG + URB602	Yes	NT	NT	248
	Exogenous Endocannabinoids+ FAAH + MGL inhibitors	2-AG +URB597+ URB602	Yes	NT	NT	248
SNL	FAAH inhibitors	URB597	Yes	Yes	NT	249
		Compound 17	Yes	NT	NT	206
		Compound 34	Yes	NT	NT	250
		JNJ-1661010	Yes	NT	NT	227