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Mini-Review

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Targeting Fatty Acid Amide Hydrolase (FAAH) to Treat Pain and Inflammation

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Abstract. The endogenous cannabinoid *N*-arachidonoyl ethanolamine (anandamide; AEA) produces most of its pharmacological effects by binding and activating CB_1 and CB_2 cannabinoid receptors within the CNS and periphery. However, the actions of AEA are short lived because of its rapid catabolism by fatty acid amide hydrolase (FAAH). Indeed, FAAH knockout mice as well as animals treated with FAAH inhibitors are severely impaired in their ability to hydrolyze AEA as well as a variety of noncannabinoid lipid signaling molecules and consequently possess greatly elevated levels of these endogenous ligands. In this mini review, we describe recent research that has investigated the functional consequences of inhibiting this enzyme in a wide range of animal models of inflammatory and neuropathic pain states. FAAH-compromised animals reliably display antinociceptive and anti-inflammatory phenotypes with a similar efficacy as direct-acting cannabinoid receptor agonists, such as Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of *Cannabis sativa*. Importantly, FAAH blockade does not elicit any apparent psychomimetic effects associated with THC or produce reinforcing effects that are predictive of human drug abuse. The beneficial effects caused by FAAH blockade in these models are predominantly mediated through the activation of CB_1 and/or CB_2 receptors, though noncannabinoid mechanisms of actions can also play contributory or even primary roles. Collectively, the current body of scientific literature suggests that activating the endogenous cannabinoid system by targeting FAAH is a promising strategy to treat pain and inflammation but lacks untoward side effects typically associated with *Cannabis sativa*.

KEY WORDS: anandamide; CB_1 cannabinoid receptor; CB_2 cannabinoid receptor; endogenous cannabinoid; fatty acid amide hydrolase (FAAH); inflammatory pain; neuropathic pain.

INTRODUCTION

The endogenous cannabinoid (EC) system is comprised of the endogenous ligands 2-arachidonoyl glycerol (2-AG) and *N*-arachidonoyl ethanolamine (anandamide; AEA) (1–3), the enzymes regulating the biosynthesis and degradation of these ligands (4), and two receptors (i.e., CB_1 and CB_2) (5,6) that bind these ECs. The CB_1 receptor is heterogeneously distributed throughout the CNS and periphery (7) and is responsible for the psychoactive effects of marijuana. Although the CB_2 receptor is primarily associated with immune cells in the periphery, it is also present on microglia cells (8) and has been identified at low levels in brainstem neurons (9).

Initially, *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) was believed to be the enzyme responsible for the biosynthesis of AEA. However, the observation that NAPE-PLD knockout mice possess wild type levels of AEA invalidated this hypothesis (10). Alternative enzymes proposed to be responsible for AEA biosynthesis include α/β-hydrolase 4 (Abh4) (11) and phospholipase C (PLC)-catalyzed cleavage of NAPE to generate a lipid, phosphoanandamide, which is subsequently dephosphorylated by phosphatases (12). Fatty acid amide hydrolase (FAAH) is the major enzyme responsible for the catabolism of AEA as well as noncannabinoid fatty acid amides (FAAs), including *N*-palmitoyl ethanolamine (PEA), *N*-oleoyl ethanolamine (OEA), oleamide (13,14), and the *N*-acyl taurines (NATs) (15).

2-AG is synthesized from phospholipid precursors by PLC and diacylglycerol lipase. Monoacylglycerol lipase (MAGL) appears to play the predominant role in 2-AG degradation (16). Cravatt's group has identified two other enzymes, α/β-hydrolase 6 and 12 (i.e., ABHD6 and ABHD12), that contribute to 2-AG metabolism in the nervous system (17). MAGL, ABHD6, and ADHD12 were determined to account for approximately 85%, 4%, and 9% of brain 2-AG hydrolysis activity, respectively. Importantly, these three enzymes display distinct subcellular distributions in the brain, suggesting that they may control different pools of 2-AG. In contrast, FAAH seems to play a negligible role in 2-AG metabolism *in vivo*.

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The EC system has been proposed to modulate a broad range of physiological processes in the CNS and periphery, including pain and inflammation, mood and anxiety disorders, neurodegenerative disorders, cancer, atherosclerosis, myocardial infarction, epilepsy, stroke, hypertension, glaucoma, obesity or metabolic syndrome, and osteoporosis (18). The presence of CB_1 receptors and ECs in CNS regions associated with pain, such as the amygdala, periaqueductal gray (PAG) and the dorsal horn of the spinal cord (7,19–21) indicates that key components of the EC system are positioned in the appropriate neuroanatomical locations to regulate pain.

Stimulation of cannabinoid receptors with Δ^9-tetrahydrocannabinol (THC), the primary active constituent of *Cannabis sativa*, and other cannabinoid receptor agonists has long been known to elicit antinociceptive and anti-inflammatory effects and is the subject of many reviews (18,22). Although cannabinoid receptor agonists produce antinociceptive and anti-inflammatory effects across a wide range of preclinical models, their psychomimetic side effects and abuse potential have dampened enthusiasm for developing drugs that act directly at CB_1 receptors. On the other hand, elevating AEA levels through genetic deletion or pharmacological inhibition of FAAH dampens pain sensitivity, without any apparent motor deficits or general pharmacological effects associated with THC. Both irreversible (e.g., URB597) and reversible (e.g., OL-135) inhibitors of FAAH produce pharmacological effects similar to those observed in FAAH (−/−) mice, including antinociception in tail immersion, hot plate, and formalin tests (14,23–25). Of great significance, URB597 lacks abuse potential in monkeys (26). Presently, MAGL (−/−) mice have yet to be created and selective inhibitors of MAGL are not available yet.[1] Thus, this mini review will focus on research investigating the role of FAAH as a target to treat pain and inflammatory disorders.

TARGETING FAAH TO TREAT INFLAMMATION AND INFLAMMATORY PAIN

There is a great deal of evidence accumulating suggesting that FAAH can be targeted to treat inflammatory disorders (28). Interestingly, rheumatoid arthritis as well as osteoarthritis patients possess elevated levels of AEA and 2-AG in the synovial fluid (29), suggesting that ECs are released on demand to offer protection in degrading arthritic connective tissues. Here, we discuss preclinical studies that have investigated the impact of blocking FAAH in common models of inflammatory pain and edema.

Carrageenan Inflammation Model

In the carrageenan model, an intraplantar injection of λ-carrageenan into the rodent hind paw elicits local edema and hyperalgesic responses to thermal and tactile stimuli. Elevating endogenous levels of AEA and other bioactive FAAs through genetic deletion or pharmacological inhibition of FAAH elicits reliable anti-edema and anti-hyperalgesic effects in the carrageenan model. FAAH (−/−) mice show phenotypic reductions in carrageenan-induced paw swelling, as do FAAH-NS mice that express FAAH exclusively on neurons (30). The FAAH (−/−) anti-edema phenotype does not appear to be mediated by CB_1 or CB_2 receptors because the appropriate selective receptor antagonists failed to normalize responses. Instead, these phenotypes may be attributed to the actions of PEA, which has anti-edematous properties when administered exogenously in mice (31) and rats (32). Conversely, systemic administration of the FAAH inhibitor URB597 reduces carrageenan-induced paw edema through a CB_2 receptor mechanism of action (33). Intraplantar administration of URB597 does not reduce edema but reduces differential weight bearing in inflamed paws (34) as well as the expansion of the nociceptor field size (35).

Complete Freund's Adjuvant Model

An intraplantar injection of complete Freund's adjuvant (CFA) into rat hind paw exacerbates immune responses at the injection site causing tissue lesions, granulomas, and localized inflammatory pain similar to that of arthritis. CFA injection is typically accompanied with both tactile and thermal hyperalgesia. Methanandamide, an AEA analog that is resistant to FAAH, reduced mechanical allodynia in CFA-treated paws, which was blocked by the CB_1 receptor antagonist AM251 (36). Administration of URB597 reduced both plantar thermal and mechanical threshold sensitivity in a dose-dependent manner, both of which were reversed by CB_1 and CB_2 receptor antagonists (37). Furthermore, chronic treatment of AM404, an inhibitor of FAAH as well as a purported AEA transporter inhibitor, blocked both thermal and mechanical hyperalgesia in inflamed paws. The anti-hyperalgesic effects of chronic AM404 were reversible by a single rimonabant injection prior to testing, suggesting a CB_1 receptor-mediated reduction in pain sensitivity (38).

Allergic Contact Dermatitis Model

The contact allergen 2,4-dinitrofluorobenzene (DNFB), which generates a specific cutaneous T cell-mediated allergic response, produces swelling of ear pinnae following repeated exposure. Research by Zimmer's group strongly suggests that this delayed-type hypersensitivity is under EC control (39). Repeated treatment of DNFB elicited a significantly increased ear swelling response in CB_1 or CB_2 receptor compromised mice. In addition, repeated administration of DNFB led to increased local levels of AEA and an increased expression of CB_2 receptor mRNA in the treated ear pinnae of wild-type mice. Finally, FAAH (−/−) mice displayed a significant decrease in DNFB-induced ear swelling. These findings, taken together, indicate that the EC system may undergo compensatory changes that dampen immunological responses to repeated exposure of a cutaneous allergen. Moreover, these findings suggest that FAAH inhibitors may represent a therapeutic approach to treat cutaneous contact hypersensitivity.

Gastrointestinal Inflammation Models

Exogenous cannabinoid receptor agonists and antagonists are well-established modulators of gastric emptying and

[1] Since the acceptance of this mini review, a research report introducing a novel and highly selective MAGL inhibitor found that inhibition of this enzyme elicited CB1 receptor mediated hypoalgesic effects in mice (27).

motility, with agonists at both CB_1 and CB_2 receptors generally inhibiting gastric motility (40). The most intense focus has been on mouse models of colonic inflammation, in which ECs have shown the clearest protective effects. Intrarectal di- or tri-nitrobenzene sulfonic acid (DNBS/TNBS) or oral dextran sulfate sodium (DSS) produces pronounced alteration in colon morphology in the form of ulceration, edema, and shortening in colon length, as well as increased MPO activity, indicating neutrophil activity. In both DNBS- and DSS-induced inflammatory models, CB_1 (−/−) mice and rimonabant-treated wild type mice show enhanced inflammation and MPO activity. Furthermore, inflammation of the colon led to a two-fold increase in CB_1-receptor expressing cells. This pattern of findings suggests that tonic endocannabinoid activity may provide protective effects against colonic inflammation (41). FAAH (−/−) mice show reductions in these inflammatory endpoints following DNBS (41). Additionally, the FAAH inhibitors, URB597 (42) and AA-5HT (43), elicited similar reductions in the DNBS and TNBS models, respectively. Likewise, VDM11, which is a substrate for FAAH and prevents AEA uptake, improved both DNBS and TNBS colon inflammation (42,43). The therapeutic effects of FAAH inhibition appear to require the activation of both CB_1 and CB_2 receptors, as CB_1 (−/−) and CB_2 (−/−) mice are resistant to the effects of these drugs (43). The above evidence has led to the implication of FAAH inhibitors and EC modulating drugs as potential treatments for painful conditions of the gastrointestinal tract and inflammatory bowel disease, including colitis, ileitis, and Crohn's disease.

NEUROPATHIC PAIN

Neuropathic pain results from nerve injury such as amputation, diabetic neuropathy, or autoimmune disease, and typical symptoms include numbness, tingling, increased sensitivity to noxious stimuli (hyperalgesia), and inappropriate perceptions of pain in response to non-noxious stimuli (i.e., allodynia). As with other types of pain, neuropathic pain is primarily treated pharmacologically, using antidepressants, opioids, and sedatives (44). Several preclinical studies have examined the role of FAAH as a target to treat neuropathic pain.

Commonly used rodent surgical procedures to induce neuropathic pain involve chronic constriction injury of the sciatic nerve (CCI), partial nerve ligation (PNG), or complete spinal nerve ligation (SNL) procedures, generally in the lumbar region. The development of neuropathic pain is thought to be driven by glial cells in the dorsal spinal cord, including microglia and astrocytes (45). Following nerve injury, glial cells become activated and produce chemokines and proinflammatory cytokines including IL-1, IL-6, and TNF-α, resulting in recruitment of immune cells to the injury site and localized inflammation, which in turn increases noxious neural stimulation and enhanced pain perception. In addition to pro-inflammatory cytokines, microglial CB_2 receptor expression is also upregulated *in vivo* following CCI (46).

In addition to increased pro-inflammatory mediators, endocannabinoids are concomitantly increased after nerve injury. In mice, CCI caused increased AEA and 2-AG levels in the spinal cord, PAG, rostral ventromedial medulla, and dorsal raphe magnus 7 days post-surgery (47). Spinal nerve ligation produced mechanical allodynia and thermal hyperalgesia in the ipsilateral hind paw, accompanied with increased AEA and 2-AG levels in the ipsilateral dorsal root ganglia (48). Interestingly, fibromyalgia patients were found to possess higher levels of circulating plasma AEA than controls (49).

Treatment with FAAH inhibitors reduces neuropathic pain in animal models of neuropathic pain. In mice, repeated oral administration of URB597 attenuated CCI-induced thermal hyperalgesia and mechanical allodynia (50). These effects were blocked by CB_1 and CB_2 receptor antagonists, suggesting that both cannabinoid receptors are working in concert. Local injection of URB597 as well as URB602, which inhibits both FAAH and MAGL, into rat paws ipsilateral to the ligated nerve, also attenuated thermal hyperalgesia and mechanical allodynia (51). Similarly, the reversible FAAH inhibitor, OL-135, reversed mechanical allodynia in a rat spinal nerve ligation model (52). This anti-allodynic effect was blocked by the CB_2 receptor antagonist, SR144528, but not by rimonabant. In addition, AM404 blocked mechanical allodynia in rats subjected to partial nerve ligation (53). This effect was blocked by a CB_1 receptor antagonist; however, CB_2 receptor antagonists were not evaluated in this study. In contrast to mice treated with FAAH inhibitors, FAAH (−/−) mice do not to display a phenotypic reduction of thermal hyperalgesia in the CCI model (24). With the exception of this neuropathic pain model, genetic deletion and pharmacological inhibition of FAAH show excellent concordance in dampening nociception in acute and inflammatory pain models. It is possible that compensatory changes occurring in FAAH (−/−) mice made them resistant to the consequences of elevated levels of AEA following neuropathic pain. Collectively, these studies indicate FAAH inhibitors are efficacious in rodent models of neuropathic pain, though the underlying mechanisms of action are dependent on species or other procedural conditions.

NONCANNABINOID RECEPTOR MECHANISMS OF ACTION

It is important to note that AEA has affinity at receptors besides CB_1 and CB_2 receptors. Also, as already described, FAAH regulates endogenous levels of AEA as well as a variety of noncannabinoid lipid signaling molecules (14,15). Thus, it should not be surprising that noncannabinoid receptor mechanisms are often found to contribute to the antinociceptive or anti-inflammatory effects caused by FAAH inhibition. The three main noncannabinoid receptors that will be reviewed here include vanilloid (TRPV1), peroxisome proliferator-activated receptors (PPAR), and opioid receptors.

Binding data as well as functional pharmacological data indicate that TRPV1 receptors contribute to the pharmacological actions of the substrates of FAAH. AEA (54) as well as the NATs (15) have been demonstrated to bind to TRPV1 receptors. The observation that the TRPV1 antagonist capsazepine blocks the thermal anti-hyperalgesic effects of intrathecal AEA in the carrageenan model (55) suggests a functional role of this "off-target." Also, CB_1 and TRPV1 receptor antagonists partially blocked the thermal analgesic effects caused by infusion of URB597 into the PAG (56) and partially blocked the anti-hyperalgesic properties of PEA in

neuropathic pain (57). Although the potency of AEA towards TRPV1 receptors is about tenfold less than that of AEA towards cannabinoid receptors, preincubation with common inflammatory mediators such as bradykinin and prostaglandin E2 shifts AEA potency for TRPV1 receptor activation into a range similar to that of cannabinoid receptors (58). However, other data suggest that AEA activation of cannabinoid and TRPV1 receptors plays opposing roles in modulating pain. Specifically, AEA stimulation of the TRPV1 receptor results in release of pro-nociceptive calcitonin gene-related peptide from sensory neurons, but CB_1 receptor activation simultaneously reduces neuronal field sensitivity and size (59).

PPARα receptors also play an important role in the analgesic and anti-inflammatory effects of URB597. For example, the PPARα receptor antagonist GW6471, but not a CB_1 receptor antagonist, blocked URB597-induced reductions in the expansion of the receptive field of spinal neurons caused by carrageenan paw inflammation (35). Similarly, GW6471 blocked the anti-hyperalgesic effects of URB597 in the carrageenan model (34). Of importance, AEA, PEA, and OEA have each been shown to bind to and activate PPARα receptors (60). Both OEA and PEA elicited anti-inflammatory effects in the carrageenan paw edema and TPA ear edema models (61). These effects were not reversed by cannabinoid receptor antagonists but were absent in PPARα (−/−) mice. These data suggest that the activation of PPARα receptors can contribute the antinociceptive effects of FAAH inhibitors.

Interactions between cannabinoid and opioid analgesia have long been an area of interest. Although naloxone failed to attenuate the analgesic effects of AEA in FAAH (−/−) mice, as evaluated in the tail immersion test (14), two other studies reported a functional role of opioid receptors in the antinociception caused by genetic or pharmacological inhibition of FAAH. First, the anti-hyperalgesic effects of OL-135 in rat spinal nerve ligation and thermal injury models were blocked by naloxone (52). Second, combined administration of URB597 and AEA elicited analgesic responses in the tail-flick test that were reversed by either systemic administration of naloxone or intrathecal administration of the kappa opioid receptor antagonist nor-BNI (62). These interesting findings should provoke additional investigation delineating the relationship between the EC and endogenous opioid systems.

CONCLUSIONS

The complementary genetic and pharmacological tools used to investigate cannabinoid receptors and enzymes regulating ECs have not only greatly increased our basic understanding of the EC system, but have also identified FAAH as a promising therapeutic target to treat a wide range of painful and inflammatory conditions. FAAH inhibitors produce anti-inflammatory and anti-hyperalgesic effects in a wide range of animal models. These beneficial effects caused by FAAH blockade are predominantly mediated through the activation of CB_1 or CB_2 receptors, though noncannabinoid mechanisms of actions can also play contributory or even primary roles. The observations that FAAH inhibitors do not elicit general cannabinoid effects and lack abuse potential are encouraging from a drug development perspective.

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