The Endogenous Cannabinoid System and Its Role in Nociceptive Behavior

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ABSTRACT: The analgesic properties of exogenous cannabinoids have been recognized for many years and suggest a regulatory role for the endogenous cannabinoid (“endocannabinoid”) system in mammalian nociceptive pathways. The endocannabinoid system includes: (1) at least two families of lipid signaling molecules, the N-acyl ethanolamines (e.g., anandamide) and the monoacylglycerols (e.g., 2-arachidonoyl glycerol); (2) multiple enzymes involved in the biosynthesis and degradation of these lipids, including the integral membrane enzyme fatty acid amide hydrolase; and (3) two G-protein coupled receptors, CB1 and CB2, which are primarily localized to the nervous system and immune system, respectively. Here, we review recent genetic, behavioral, and pharmacological studies that have tested the function of the endocannabinoid system in pain sensation. Collectively, these investigations support a role for endocannabinoids in modulating behavioral responses to acute, inflammatory, and neuropathic pain stimuli.

INTRODUCTION

The medicinal properties of Δ9-tetrahydrocannabinol (THC), the active component of marijuana, have been recognized for centuries (Mechoulam, 1986); however, only recently have we gained insights into the molecular mechanism of action of this powerful pharmacological agent. In the early 1990s, two G-protein-coupled receptors (GPCRs), CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993), were characterized that recognize THC and other cannabinoid agonists. The CB1 and CB2 receptors share 50% sequence identity, and are most strongly expressed in the nervous system and immune system, respectively. CB receptors also share low, but significant homology with other GPCRs that recognize lipids, such as the edg receptors, which bind lysophosphatidic acid and sphingosine 1-phosphate (Fukushima et al., 2001). Subsequent pharmacological studies with receptor-selective agonists and receptor knockout (−/−) mice have confirmed that the majority of the neurobehavioral effects of cannabinoids, which include hypomotility, hypothermia, catalepsy, and analgesia, are mediated by the CB1 receptor (Ledent et al., 1999; Zimmer et al., 1999).
The pursuit of endogenous ligands for CB receptors ("endocannabinoids") has resulted in the identification of two major classes of lipids that activate these receptors: the N-acyl ethanolamines (NAEs) and the monoacylglycerols (MAGs) (Di Marzo et al., 1999). The most well-studied NAE endocannabinoid is N-arachidonoyl ethanolamine, or anandamide (Devane et al., 1992) (Fig. 1), which activates both the CB1 and CB2 receptors. Of the MAGs, 2-arachidonoylethanolglycerol (2-AG) represents the most potent agonist for CB receptors (Mechoulam et al., 1995; Stella et al., 1997) (Fig. 1). In contrast, saturated or mono-unsaturated NAEs and MAGs are mostly inactive as CB receptor ligands. Thus, a general structure–activity relationship has emerged in which both the CB1 and CB2 receptors prefer binding amide/ester lipids that possess a polyunsaturated (arachidonoyl) acyl chain. Nonetheless, it is noteworthy that several "non-cannabinoid" NAEs also appear to serve as endogenous signaling lipids, including N-palmitoyl ethanolamine (PEA) and N-oleoyl ethanolamine (OEA), which modulate pain sensation (Jaggar et al., 1998; Calignano et al., 1998) and feeding (Rodriguez de Fonseca et al., 2001), respectively. In addition, the fatty acid primary amide oleamide has been shown to modulate sleep and reduce body temperature in rats (Cravatt et al., 1995; Mechoulam et al., 1997; Basile et al., 1999).

Before discussing the relationship among CB receptors, their natural ligands, and pain, we will briefly review our current understanding of the enzymatic mechanisms for NAE and MAG biosynthesis and degradation, as these metabolic pathways have provided important new targets for testing the role that endocannabinoids play in nociception, as well as other physiological processes. NAEs appear to be produced by a two-step enzymatic pathway involving the sequential action of a calcium-dependent transacylase that catalyzes the formation of N-acylphosphatidylethanolamines (NAPEs) and a phospholipase D that hydrolyzes NAPEs to release NAEs (Sugiura et al., 1996; Cadas et al., 1997) [Fig. 2(A)]. Notably, a candidate phospholipase D that selectively hydrolyzes NAPEs was recently purified and molecularly characterized from bovine brain (Okamoto et al., 2004). The identity of the transacylase enzyme remains unknown, although its calcium dependence suggests that NAE production in the nervous system is restricted to sites of neuronal activation. The magnitude and duration of NAE signaling is tightly controlled by the uptake and catabolism of these lipids (Giuffrida et al., 2001; Cravatt and Lichtman, 2002). A protein-mediated transporter has been proposed to participate in the cellular uptake of anandamide (Beltramo et al., 1997), although the existence of this transport protein is still controversial (Glaser et al., 2003). Evidence in support of an anandamide transporter includes the temperature dependency, saturability, selective inhibition, and substrate specificity of this process. On the other hand, these properties have also been suggested to be consistent with other models for the cellular uptake of anandamide (Patricelli and Cravatt, 2001). In particular, studies supporting a specific transporter have been criticized because anandamide accumulation is assessed over prolonged periods of time when metabolism and intracellular sequestration become relevant factors (Glaser et al., 2003). Moreover, most purported transport inhibitors also inhibit FAAH, and fail to inhibit anandamide uptake at short time points. For a further discussion see a recent review by Hillard

![Figure 1](image1.png) Chemical structures of the two major endocannabinoids anandamide and 2-arachidonoylglycerol.

![Figure 2](image2.png) Enzymes involved in the biosynthesis (A) and degradation (B) of anandamide and other members of the N-acyl ethanolamine (NAE) family of endogenous lipids.
Once NAEs have entered cells (via a protein-assisted process, simple diffusion, or both), they are rapidly degraded by the integral membrane enzyme fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), which hydrolyzes these lipids to their corresponding acids [Fig. 2(B)]. The central role that FAAH plays in controlling NAE levels in vivo was confirmed by the analysis of FAAH(−/−) mice (Cravatt et al., 2001), which possess greatly elevated endogenous levels of NAEs in several brain regions, including hippocampus, cortex, and cerebellum (Clement et al., 2003). Notably, as will be discussed in more detail below, FAAH(−/−) mice have served as a valuable animal model in which the impact of tonically elevated endocannabinoid levels on a variety of neurobehaviors, including nociception, can be examined.

Over the past few years, key insights into the mechanisms for MAG biosynthesis and degradation have also been achieved. 2-MAGs appear to be produced in the nervous system via the enzymatic hydrolysis of the sn-1 acyl chain of diacylglycerol (DAG) lipids [Fig. 3(A)], and recently, the first sn-1 selective DAG lipase was molecularly characterized (Bisogno et al., 2003). Similar to NAEs, the termination of MAG signals is proposed to occur via the sequential cellular uptake and degradation of these lipids (Dinh et al., 2002). Initially, MAG degradation was suggested to be mediated by FAAH, which hydrolyzes these lipids at a rate similar to fatty acid amides (Goparaju et al., 1998). However, the absolute rates of MAG hydrolysis in brain extracts far exceed those for NAEs (Lichtman et al., 2002), suggesting that enzymes other than FAAH are primarily responsible for catabolizing MAGs in vivo. Consistent with this notion, mice treated with FAAH inhibitors (Kathuria et al., 2003), or FAAH(−/−) mice (our unpublished findings) show no changes in MAG brain levels. One candidate enzyme responsible for MAG degradation in vivo is the serine lipase monoacylglycerol lipase (Dinh et al., 2002) [Fig. 3(B)], which is expressed in the brain and a variety of peripheral tissues. Nonetheless, a definitive understanding of the relative contribution made by the cloned sn-1 selective DAG lipases and MAG lipase to the biosynthesis and degradation of MAGs, respectively, will require an analysis of the neurochemical consequences of the genetic and/or chemical inactivation of these enzymes in vivo.

In recent years, the endocannabinoid system has been implicated in a myriad behavioral processes, including memory (Varvel and Lichtman, 2002; Marusicano et al., 2002), emotional state (Kathuria et al., 2003), feeding (Di Marzo et al., 2001), inflammation (Maccarrone et al., 2002), and nociception (Calignano et al., 1998; Walker et al., 1999; Cravatt et al., 2001). Here, we will focus on emerging roles for the endocannabinoid system in the regulation of pain behavior. Collectively, the studies highlighted in this review suggest that the endocannabinoid system may operate at multiple levels, both central and peripheral, to mitigate responses to a variety of acute and chronic nociceptive stimuli.

**The CB1 Receptor and Its Role in Nociception**

Pharmacology studies in rodents have provided a preponderance of evidence that activation of the CB1 receptor by exogenously applied agonists reduces pain sensitivity in a variety of nociceptive assays. These findings have been the subject of several recent reviews (Martin and Lichtman, 1998; Pertwee, 2001; Rice, 2001; Walker and Huang, 2002; Hohmann, 2002; Goya et al., 2003) and will therefore only be briefly discussed here.

In rodents, THC and other CB1 agonists have been known for many years to promote analgesia in a number of acute pain models, including the tail-flick and hot-plate tests of thermal nociception, as well as the acetic acid writhing and formalin tests of tonic, noxious pain (Martin and Lichtman, 1998). More recently, CB1 agonists have also been demonstrated to suppress hyperalgesia in chronic pain models. The synthetic CB1 agonist WIN 55,212-2 has been found to produce antihyperalgesic activity in rat models of neuropathic pain (Herzberg et al., 1997; Monhemius...
et al., 2001; Bridges et al., 2001; Fox et al., 2001; Lim et al., 2003; Costa et al., 2004), apparently through both central (Monhemius et al., 2001; Fox et al., 2001; Lim et al., 2003) and peripheral (Fox et al., 2001) mechanisms. Additionally, CB1 agonists display antihyperalgesic properties in the Freund’s adjuvant model of persistent inflammatory pain (Smith et al., 1998; Martin et al., 1999). Collectively, these studies support a role for the CB1 receptor in modulating both acute and chronic pain stimuli.

As can be gleaned from the studies described above, exogenous CB1 agonists are capable of promoting analgesia by acting at several sites along neural pathways for pain transmission. Indeed, the activation of peripheral (Richardson et al., 1998b; Calignano et al., 1998; Fox et al., 2001), spinal (Yaksh, 1981; Lichtman and Martin, 1991; Lichtman et al., 1992; Smith and Martin, 1992; Smith et al., 1998; Martin et al., 1999; Martin et al., 1999; Lim et al., 2003), and supraspinal (Lichtman and Martin, 1991, 1997; Smith and Martin, 1992; Martin et al., 1993, 1995; Lichtman et al., 1996; Monhemius et al., 2001) CB1 receptors has been shown to independently reduce nociception. Consistent with these pharmacological findings, immunohistochemical studies indicate that the CB1 receptor is expressed at high levels in a variety of peripheral (Sanudo-Pena et al., 1999; Ahluwalia et al., 2000) and central [both spinal and supraspinal (Tsou et al., 1998; Farquhar-Smith et al., 2000)] neurons that participate in pain perception. Finally, electrophysiological studies have provided evidence that CB1 receptor agonists modulate both spinal (Hohmann et al., 1995, 1998; Strangman et al., 1998; Strangman and Walker, 1999; Drew et al., 2000; Chapman, 2001; Kelly and Chapman, 2001) and supraspinal (Martin et al., 1996; Meng et al., 1998) neural circuits that transmit nociceptive signals.

In summary, based on a variety of pharmacological, anatomical, and electrophysiological investigations, the CB1 receptor system seems poised to play a fundamental role in regulating pain behavior. The pervasive antinociceptive properties of CB1 agonists would seem to suggest that such agents may be of great clinical utility for treating a variety of pain disorders. However, the analgesic properties of CB1 agonists have, to date, proven extremely difficult to separate from a number of unwanted side effects produced by these agents, including motility and cognitive defects that currently limit their therapeutic application (Table 1). Regardless of the clinical potential of CB1 agonists, it is important to recognize that the effects of these agents on pain sensation do not directly address the question of whether endogenously produced cannabinoids also modulate nociception, either tonically or in response to injury/damage. We will return to this important subject in a later section of this review.

The CB2 Receptor and Its Role in Nociception

Originally, given its restricted expression in the immune system, the CB2 receptor was not anticipated to play a role in nociception. However, several recent studies have provided provocative evidence that activation of the CB2 receptor mitigates pain in response to a variety of acute and chronic stimuli (Malan et al., 2002). For example, the CB2-selective agonist HU-308 was found to reduce pain behavior in the late phase of the formalin test (Hanus et al., 1999). Likewise, a second CB2-selective agonist AM1241 fully reversed inflammatory hyperalgesia and edema in rats administered carrageenan into the hind paw (Nackley et al., 2003; Quartilho et al., 2003) and also blocked hyperalgesia elicited by capsaicin (Hohmann et al., 2004). In these studies, AM1241 was effective when injected into the ipsilateral, but not contralateral paw, suggesting a local mechanism of action. AM1241 was also recently found to inhibit tactile and thermal hypersensitivity in a nerve ligation rodent model of neuropathic pain (Ibrahim et al., 2003). Curiously, however, AM1241 (Malan et al., 2001; Ibrahim et al., 2003), but not HU-308 (Hanus et al., 1999) also produced analgesia in response to acute thermal and
mechanical stimuli, suggesting a possible role for CB2 receptors in regulating acute (as well as injury-induced) nociception.

In all of the models described above, the antinociceptive effects of CB2 agonists were reversed by CB2 receptor antagonists, but not CB1 receptor antagonists, and occurred in the absence of any detectable effects on motility, body temperature, or cognition that are typically observed with CB1 agonists (Table 1). These findings suggest that activation of the CB2 receptor may promote analgesia without the unwanted (e.g., psychoactive) side effects that accompany stimulation of the CB1 receptor. Also potentially consistent with this notion, THC was shown to induce analgesia in the tail flick test, but not hypothermia or catalepsy in CB1(−/−) mice (Ledent et al., 1999; Zimmer et al., 1999), which might suggest the specific involvement of CB2 receptors in pain sensation. Still, the fact that THC produces residual analgesia in CB(−/−) mice does not necessarily imply the activation of CB2 receptors, as other receptor systems may be involved. Further complicating the interpretation of this study, the analgesic effects of the cannabinoid HU-210 were completely annihilated in CB1(−/−) mice (Zimmer et al., 1999), and THC treatment failed to elicit analgesia in other pain tests in these animals (e.g., hot plate; Ledent et al., 1999; Zimmer et al., 1999).

Several intriguing questions regarding the relationship between CB2 receptors and nociception remain unanswered. For example, why does activation of the CB2 receptor, which is nearly exclusively localized to immune cells, inhibit noninflammatory acute and neuropathic pain sensation? A recent in situ hybridization study examining CB2 receptor expression in the rat spinal cord under normal, inflammatory, and neuropathic pain states may shed some light on this issue (Zhang et al., 2003). In this study, peripheral nerve injury, but not peripheral inflammation, was found to induce CB2 receptor expression in specific cell types within the lumbar spinal cord that appear to correspond to activated microglia. These findings suggest that CB2 receptor-expressing microglia may specifically modulate the spinal processing of nerve injury-induced pain signals. Although this model is quite provocative, it does not explain all of the data obtained to date with CB2 agonists. For example, if CB2 receptors are only expressed in the spinal cord in response to nerve injury, why do at least some CB2 agonists reduce acute pain responses in uninjured mice? One possible explanation that has been put forth suggests that CB2 agonists may decrease the activity of primary afferent neurons by inhibiting the release of sensitizing substances (e.g., histamine, prostaglandins) from neighboring mast and immune cells (Malan et al., 2001).

Regardless of the precise mechanism(s) involved, exogenous CB2 agonists have clearly been shown to reduce pain responses to a variety of acute and chronic stimuli. Still, as was the case for the CB1 receptor system, whether endogenous ligands for the CB2 receptor also modulate nociception remains unknown. We will now review a series of recent pharmacological and genetic studies that have attempted to address the role that the endocannabinoid system plays in regulating pain behavior.

### The Endocannabinoid System and Its Role in Nociception

Although the activation of CB1 and CB2 receptors by exogenously applied agonists promotes analgesia in a number of acute and chronic pain models, these pharmacological studies do not directly address the role that endogenous ligands for these receptors (“endocannabinoids”) play in nociceptive processes. To test whether the endocannabinoid system modulates pain behavior, researchers have employed both CB receptor antagonists and transgenic mice in which key protein components of the endocannabinoid system have been specifically deleted.

#### The Effects of CB Receptor Antagonists on Nociception

The effects of CB1 and CB2 antagonists on mammalian pain responses have provided, at best, equivocal findings. For example, initial studies with the CB1 antagonist SR141716A showed that this agent did not alter acute pain sensitivity in the tail flick test in rats (Rinaldi-Carmona et al., 1994). Subsequent work in mice has supported this finding, where SR14176A was found to have no effect on pain sensitivity in either the tail immersion or hot plate tests (Cravatt et al., 2001; Lichtman et al., 2004b). In contrast, SR141716A has been reported in other studies to produce hyperalgesia in the tail flick (Costa and Colleoni, 1999) and hot plate (Richardson et al., 1998a) test in rats. In the formalin test of persistent pain, a similar set of conflicting results has emerged for CB1 antagonists, as initial studies described a hyperalgesic activity for these agents (Calignano et al., 1998; Strangman et al., 1998), but subsequent reports have failed to confirm these findings (Beaulieu et al., 2000; Lichtman et al., 2004b). Interestingly, CB1 antagonists have been reported to reverse the antinociceptive activity of the cyclooxygenase inhibitor flurbiprofen in the formalin test, suggesting an interplay between the endocannabinoid and prostaglandin systems in vivo (Ates et al., 2003).
Fewer studies have been conducted to date examining the effects of CB2 receptor antagonists on nociception. Notably, oral administration of two CB2 antagonists JTE907 and SR144528 elicited antinociceptive effects in the carrageenan mouse model (Iwamura et al., 2001). Contradictory to these findings, however, SR144528 has also been reported to enhance carrageenan-induced edema and hyperalgesia (Clayton et al., 2002). Likewise, in the formalin test, CB2 antagonists have been found to either produce hyperalgesia (Calignano et al., 1998) or have no effect (Beaulieu et al., 2000; Lichtman et al., 2004b) on pain sensation. Thus, as has been the case with the CB1 receptor antagonists, experiments with CB2 receptor antagonists have provided conflicting data regarding a potential role for the endocannabinoid system in nociception. Further complicating studies with CB receptor antagonists is the fact that these agents are also inverse agonists (Landsman et al., 1997; Portier et al., 1999), meaning that they may produce effects through their respective receptors without necessarily disrupting the activity of endogenous agonists (endocannabinoids).

The Effects of CB1 Receptor Gene Disruption on Nociception. Studies of pain perception in CB1 receptor knockout [CB1(−/−)] mice have also yielded ambiguous results. For example, in one study, CB1(−/−) mice were found to show hypoalgesic responses in both the hot plate and formalin tests, but no change in nociceptive behavior in the tail-flick assay (Zimmer et al., 1999). These findings are somewhat counterintuitive because inactivation of the CB1 receptor would have been anticipated, based on previous studies with CB1 agonists, to promote increases in pain sensitivity, rather than the opposite. However, it is possible that the constitutive inactivation of CB1 receptors leads to either compensatory changes in the nervous system that account for the unexpected pain responses of CB1(−/−) mice or other behavioral changes that interfere with the measurement of nociception. On this note, CB1(−/−) mice also exhibited decreases in general locomotor activity and reduced mobility in the ring test, which may reflect disrupted motor function that could confound the interpretation of findings from hot plate and formalin tests. In contrast to these results, a second analysis of CB1(−/−) mice found that these animals displayed no significant differences in nociceptive behavior in hot plate, tail-immersion, writhing, and tail-pressure tests (Liedet al., 1999). Interestingly, however, these animals were found to show reduced antinociception following a forced swim test (Valverde et al., 2000), suggesting a role for the endocannabinoid system in modulating stress-induced analgesia. Finally, a recent third study of CB1(−/−) mice reported normal thermal pain sensitivity, but increased tactile sensitivity in CB1(−/−) mice (Ibrahim et al., 2003). Although the basis for these apparently conflicting findings remains unclear, one possible explanation is that the CB1(−/−) mice used in each study were of distinct genetic background (C57Bl/6, CD1, and 129/SvJ, respectively). Indeed, the baseline pain sensitivity of different inbred strains of mice has been shown to vary greatly (Mogil et al., 1999), and therefore could influence the effect of CB1 receptor inactivation.

In contrast to studies of CB1(−/−) mice, where the CB1 receptor has been constitutively deleted, the transient “knock-down” of CB1 receptors in the spinal cord by antisense methods has been reported to augment pain behavior (Richardson et al., 1998a; Dogrul et al., 2002), thereby providing evidence in favor of a tonic endocannabinoid influence over nociception in this region of the CNS. Consistent with this notion, intrathecal administration of SR141716A has been found to increase thermal pain sensitivity (Richardson et al., 1998a), and facilitate the nociceptive responses of dorsal horn neurons to acute pain stimuli (Chapman, 1999).

Several additional lines of indirect evidence suggest a role for the endocannabinoid system in modulating chronic pain sensitivity. For example, the CB1 receptor has been shown to be upregulated in both the thalamus (Siegel et al., 2001) and spinal cord (Lim et al., 2003) in rodent models of neuropathic pain. In the latter example, treatment with a MAP kinase inhibitor blocked both CB1 upregulation and the enhanced efficacy of CB1 agonists in reducing hyperalgesia, suggesting that the elevated levels of CB1 receptors following nerve injury may mediate the analgesic effects of cannabinoids on neuropathic pain. A more recent study showed that CB2 receptors are also upregulated in the spinal cord of rats following peripheral nerve injury (Zhang et al., 2003). Notably, chronic inflammatory pain has also been reported to alter CB1 receptor activity (Martin et al., 1999), suggesting that the endocannabinoid system may play a general role in the regulation of persistent pain states.

Finally, anandamide has been shown using in vitro models to also stimulate the vanilloid receptor VR1, now called the TRPV1 receptor (Zylmunt et al., 1999), suggesting that endocannabinoids may also affect pain transmission through noncannabinoid sites of action. The issue of endocannabinoids acting at the TRPV1 receptor, as well as the TRPV3 receptor, has recently been reviewed elsewhere (Di Marzo et al., 2002; Nilius et al., 2004).

Collectively, these data suggest that an endocan-
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In each of these cases, the analgesic phenotype of FAAH(−/−) mice was reversed by SR141716A, consistent with elevated anandamide levels promoting these behavioral effects by acting on CB1 receptors.

Interestingly, FAAH(−/−) mice have also been shown to exhibit reduced hyperalgesia and paw edema in the carrageenan model of inflammatory pain, but these responses were not sensitive to SR141716A and only partially mitigated by the CB2 antagonist SR144528 (Lichtman et al., 2004b). These findings suggest that fatty acid amides other than anandamide may be responsible for the antihyperalgesic and antiinflammatory phenotypes observed in FAAH(−/−) mice in the carrageenan test. On this note, the NAE, N-palmitoyl ethanolamine, which is also dramatically upregulated in tissues from FAAH(−/−) mice, has been shown to possess analgesic (Calignano et al., 1998; Jaggar et al., 1998) and antiinflammatory properties (Conti et al., 2002), possibly by acting on an uncharacterized CB2-like receptor, and therefore represents an attractive candidate for mediating these effects in FAAH(−/−) mice. Regardless of the specific fatty acid amide(s) involved, the recent determination that FAAH(−/−) mice also show reduced inflammation in a dinitrobenzene sulfonic acid model of colitis (Massa et al., 2004) suggests that these animals may display a general antiinflammatory phenotype.

In contrast to their analgesic behavior in acute and inflammatory pain models, FAAH(−/−) mice were not found to show altered thermal pain responses in the chronic constrictive injury model of neuropathic pain (Lichtman et al., 2004b). Although this finding might suggest that endocannabinoids (or at least the fatty acid amide subclass of endocannabinoids) do not substantially influence chronic pain states, other interpretations are possible. For example, the constitutive elevation of endocannabinoids in the nervous system of FAAH(−/−) mice may result in desensitization of CB receptor activity in chronic pain models. Somewhat consistent with this idea, both CB1 (Sieging et al., 2001; Lim et al., 2003) and CB2 (Zhang et al., 2003) receptors are upregulated in the spinal cord in response to nerve injury, indicating that neuropathic pain conditions can alter the state of the endocannabinoid system.

To complement studies of FAAH(−/−) mice, researchers have also examined the pharmacological activity of FAAH inhibitors, which more directly test the behavioral effects that result from the transient elevation of fatty acid amides in vivo. For example, two recent studies have described novel inhibitors of FAAH that promote analgesia in rodents. Scientists at Bristol-Meyers Squibb described in 2002 and 2003...
patients a series of biaryl carbamate and oxime-carbamate inhibitors of FAAH that showed antinociceptive activity in several rodent pain models, including both phases of the formalin test, the carrageenan paw inflammation test, and the Chung model of neuropathic pain (Sit and Xie, 2002, 2003). Similarly, Kathuria and colleagues generated a distinct series of potent carbamate inhibitors of FAAH that, when administered to mice, caused: (1) significant elevations in the brain levels of anandamide, and (2) CB1-dependent antinociception in the hot-plate test (Kathuria et al., 2003). Interestingly, in this latter study, the authors also reported a strong CB1-dependent anxiolytic activity for FAAH inhibitors, indicating that the endocannabinoid system modulates other behavioral processes in addition to nociception. Finally, in contrast to the carbamate inhibitors of FAAH, which likely inhibit the enzyme irreversibly, the first class of highly potent and selective reversible FAAH inhibitors was recently reported by Boger and colleagues (Boger et al., 2000; Leung et al., 2003). These reversible FAAH inhibitors also promote CB1-dependent analgesia in multiple pain models (Lichtman et al., 2004a).

In summary, studies to date using FAAH(−/−) mice and FAAH inhibitors support a role for endocannabinoid signaling in the modulation of both acute and chronic pain sensitivity, and suggest that FAAH may be an important new drug target for the treatment of pain, as well as other neural disorders. Indeed, it is noteworthy that either the chemical or genetic inactivation of FAAH produces a provocative subset of the behavioral effects observed with CB1 agonists, promoting analgesia in a variety of pain models without also causing hypomotility, hypothermia, or catalepsy (Table 1). These findings suggest that FAAH inhibition may increase endocannabinoid tone in selective neural circuits associated with nociception and produce pain relief without the undesired side effects that accompany global activation of the CB1 receptor.

CONCLUSIONS AND THERAPEUTIC IMPLICATIONS

Although the analgesic properties of exogenous cannabinoids, like THC, have been appreciated for many years, much less is known about the role that the endogenous cannabinoid system plays in nociception. In this review, we have attempted to summarize a large body of recent work aimed at addressing this important problem. Overall, studies with CB receptor antagonists and knockout mice have produced equivocal results regarding the presence of an endocannabinoid tone that constitutively modulates pain perception. Although the variable results obtained in these investigations could potentially be accounted for by differences in experimental procedures, such as the choice of pain model, route of drug administration, and/or background strain of transgenic mouse, one would still likely conclude from these studies that endocannabinoids, at best, function as weak modulators of acute pain responses in vivo. On the other hand, considering that both the CB1 and CB2 receptors are upregulated in discrete regions of the CNS in response to inflammation and/or nerve injury, it is intriguing to speculate that endocannabinoids may exert a preferential influence over chronic pain states. Further studies aimed at probing the role of endocannabinoids in inflammatory and neuropathic pain are required to address this interesting possibility.

Although endocannabinoids, at their natural concentrations in vivo, may exhibit a rather tempered influence over pain pathways, the elevation of these signaling lipids in the nervous system by, for example, the pharmacological and/or genetic inactivation of FAAH, has provided provocative evidence that increases in endocannabinoid tone can promote significant analgesia in a number of acute and chronic pain models. Collectively, these findings suggest that the regulation of endocannabinoid metabolism, either by endogenous or exogenous factors, is one means by which to alter the relative impact that these signaling lipids have on nociceptive responses. Still, further studies are needed to clarify the role that the endocannabinoid system plays in specific pain states. For example, why do FAAH inhibitors reduce hyperalgesia in response to nerve injury, but FAAH(−/−) mice fail to show altered pain responses in similar neuropathic pain models? One possibility is that the constitutively elevated CNS levels of endocannabinoids in FAAH(−/−) mice lead to compensatory changes in certain nociceptive pathways that mitigate the impact of these signaling lipids on chronic pain responses. Studies in which FAAH has been inactivated only address a subset of the features of the endocannabinoid system, and leave several important questions unanswered. For example, would reductions in endocannabinoid biosynthesis promote hyperalgesia (or other neurobehavioral effects)? Similarly, what would be the physiological effects of altering the levels of other endocannabinoids, like 2-AG? As the identities of additional protein components of endocannabinoid pathways become clear, new animal models should emerge that further refine our understanding of this complex lipid signaling system.

Finally, it is worth considering the therapeutic implications of the studies described in this review.
Although CB1 agonists have long been considered as potential drugs for the treatment of pain, these agents also produce substantial psychotropic effects that limit their general clinical utility. In this regard, CB2 agonists and inhibitors of FAAH represent attractive alternatives to CB1 agonists, as these agents promote analgesia without causing significant locomotor or cognitive side effects (Table 1). Future efforts to target novel components of the endocannabinoid system, either through selectively activating its peripheral pathways (e.g., CB2 receptor agonists) or augmenting its natural signaling power (e.g., FAAH inhibitors) may achieve the long-desired goal of exploiting the therapeutic potential of the endocannabinoid system without enduring its adverse properties.

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