The Endogenous Cannabinoid System and Its Role in Nociceptive Behavior

Benjamin F. Cravatt,[1] Aron H. Lichtman[2]

[1] The Skaggs Institute for Chemical Biology and Departments of Cell Biology and Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, California 92037

[2] Department of Pharmacology and Toxicology, P.O. Box 980613, Virginia Commonwealth University, Richmond, Virginia 23298

Received 1 March 2004; accepted 17 May 2004

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.
 J Neurobiol 61: 149–160, 2004
Keywords: **cannabinoid; endocannabinoid; pain; fatty acid amide hydrolase; CB1 receptor; CB2 receptor; agonist; antagonist; inhibitor**

INTRODUCTION

The medicinal properties of Δ^9-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).

Correspondence to: B.F. Cravatt (cravatt@scripps.edu).
Contract grant sponsor: the National Institute of Drug Abuse, of the National Institutes of Health; contract grant numbers: 015197 and 017259 (to B.F. Cravatt), and 03672 and 09789 (to A.H. Lichtman).
Contract grant sponsor: the Helen L. Dorris Institute for the Study of Neurological and Psychiatric Disorders in Children and Adolescents.
Contract grant sponsor: The Skaggs Institute for Chemical Biology.

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/neu.20080

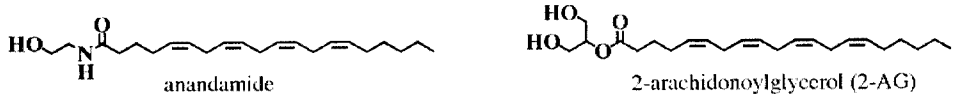

Figure 1 Chemical structures of the two major endocannabinoids anandamide and 2-arachidonoylglycerol.

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-arachidonoylglycerol (2-AG) represents the most potent agonist for CB receptors (Mechoulam et al., 1995; Stella et al., 1997) (Fig. 1). In contrast, saturated or monounsaturated 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 "noncannabinoid" 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

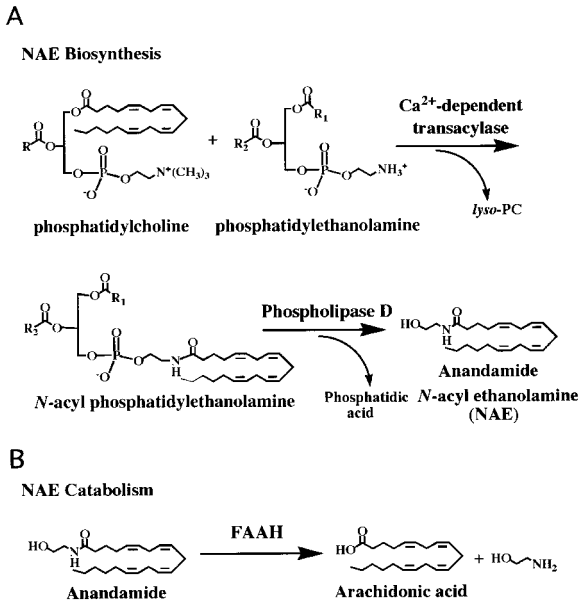

Figure 2 Enzymes involved in the biosynthesis (A) and degradation (B) of anandamide and other members of the *N*-acyl ethanolamine (NAE) family of endogenous lipids.

and Jarrahian (2000). 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

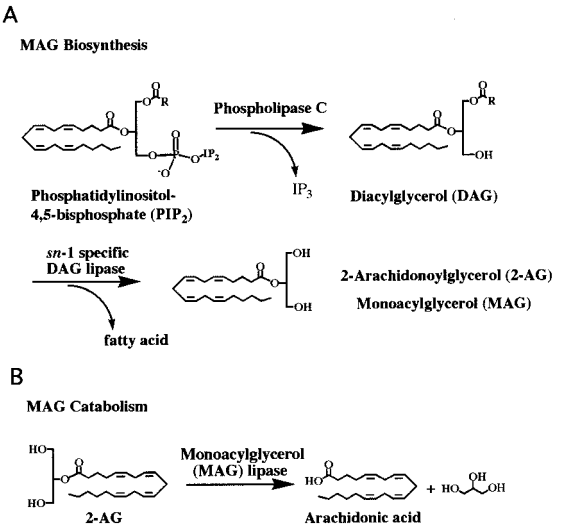

Figure 3 Enzymes involved in the biosynthesis (A) and degradation (B) of 2-arachidonoyl glycerol (2-AG) and other members of the 2-monoacylglycerol (MAG) family of endogenous lipids.

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; Marsicano 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

Table 1 A Comparison of the Antinociceptive Effects and Side Effects Produced by CB Receptor Agonists vs. the Genetic (−/−) or Chemical (Inhibitors) Inactivation of FAAH

	Antinociceptive Effects in Representative Pain Tests					Side Effects		
	Acute Thermal	Acute Mechanical	Noxious	Inflammatory	Neuropathic	Hypomotilty	Hypothermia	Catalepsy
CB1 agonist	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CB2 agonist	Yes/No	Yes	Unknown	Yes	Yes	No	No	No
FAAH(−/−) mice	Yes	Unknown	Yes	Yes	No	No	No	No
FAAH inhibitors	Yes	Unknown	Yes	Yes	Yes	No	No	No

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 (Beaulieul 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 antiinflammatory 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 (Beaulieul et al., 2000; Lichtman et al., 2004b) on pain sensation. Thus, as has been the case with 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; Iwamura et al., 2001), 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 (Ledent et 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 show 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 (Siegling 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 (Zygmunt 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-

nabinoid tone may exist that both regulates and is regulated by pain signaling pathways *in vivo*. However, these studies do not directly address the potential role that endocannabinoids themselves play in pain perception, which is a particularly important issue given that both the CB1 and CB2 receptors possess significant tonic activity, meaning that antagonists of these receptors may exhibit inverse agonist properties *in vivo* (i.e., produce behavioral effects independent of blocking the function of an endogenous CB ligand) (Shire et al., 1999). To more directly test the role of endocannabinoids in nociception, one interesting study showed that electrical stimulation of the dorsal periaqueductal gray (PAG) led to the concurrent production of SR141716A-sensitive analgesia and the release of anandamide in this brain region (Walker et al., 1999). Notably, injections of formalin into the hindpaws also elevated anandamide in the PAG, suggesting that this endocannabinoid is upregulated by peripheral pain stimuli. To further examine a potential functional link between endocannabinoids and nociception, researchers have generated pharmacological agents and animal models that can be used to specifically test the activity of endogenous ligands for CB receptors *in vivo*. These research efforts will be discussed in the next section.

Blocking Anandamide Metabolism* In Vivo *and its Effect on Nociception. If an "endocannabinoid tone" exists that regulates pain transmission, one might expect that shifts in the strength of this tone would alter the magnitude of influence that the endocannabinoid system exhibits over nociceptive responses. One powerful strategy to upregulate endocannabinoid tone is through the genetic and/or pharmacological inactivation of FAAH, an integral membrane enzyme that shows a complementary localization to the CB1 receptor in the nervous system (Egertova et al., 1998) and terminates anandamide signaling by degrading this lipid to arachidonic acid and ethanolamine (Cravatt et al., 1996). The central role that FAAH plays in regulating anandamide signaling *in vivo* has been exemplified by analyses of FAAH(−/−) mice (Cravatt et al., 2001), which possess dramatically elevated levels of anandamide (and other fatty acid amides) in several brain regions (Clement et al., 2003) and the spinal cord (our unpublished findings) of FAAH(−/−) mice. Interestingly, these constitutive changes in endocannabinoid neurochemistry in FAAH(−/−) mice correlate with an analgesic phenotype observed in several pain models. For example, FAAH(−/−) mice exhibit reduced pain responses in the tail-immersion, hot plate, and formalin tests (both phases) (Cravatt et al., 2001; Lichtman et al., 2004b). 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 (Siegling 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

patents 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 normal 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.

REFERENCES

Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. 2000. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience 100:685–688.

Ates M, Hamza M, Seidel K, Kotalla CE, Ledent C, Guhring H. 2003. Intrathecally applied fluribiprofen produces an endocannabinoid-dependent antinociception in the rat formalin test. Eur J Neurosci 17:597–604.

Basile AS, Hanus L, Mendelson WB. 1999. Characterization of the hypnotic properties of oleamide. Neuroreport 10:947–951.

Beaulieul P, Bisogno T, Punwar S, Farquhar-Smith WP, Ambrosino G, Di Marzo V, Rice AS. 2000. Role of endogenous cannabinoid system in the formalin test of persistent pain in the rat. Eur J Pharmacol 396:85–92.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277:1094–1097.

Bisogno T, Howell F, WIlliams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163:463–468.

Boger DL, Sato H, Lerner AE, Hedrick MP, Fecik RA, Miyauchi H, Wilkie GD, Austin BJ, Patricelli MP, Cravatt BF. 2000. Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. Proc Natl Acad Sci USA 97:5044–5049.

Bridges D, Ahmand K, Rice AS. 2001. The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br J Pharmacol 133:586–594.

Cadas H, di Tomaso E, Piomelli D. 1997. Occurence and biosynthesis of endogenous cannabinoid precursor, N-arachidonyl phosphatidylethanolamine, in rat brain. J Neurosci 17:1226–1242.

Calignano A, La Rana G, Giuffrida A, Piomelli D. 1998. Control of pain initiation by endogenous cannabinoids. Nature 394:277–281.

Chapman V. 1999. The cannabinoid CB1 receptor antagonist, SR141716A, selectively facilitates nociceptive responses of dorsal horn neurones in the rat. Br J Pharmacol 127:1765–1767.

Chapman V. 2001. Functional changes in the inhibitory effect of spinal cannabinoid receptor activation in nerve injured rats. Neuropharmacology 41:870–877.

Clayton N, Marshall FH, Bountra C, O'Shaughnessy CT. 2002. CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. Pain 96:253–260.

Clement AC, Hawkins EG, Lichtman AH, Cravatt BF. 2003. Increased seizure susceptibility and pro-convulsant activity of anandamide in mice lacking fatty acid amide hydrolase. J Neurosci 23:3916–3923.

Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G. 2002. Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. Br J Pharmacol 135:181–187.

Costa B, Colleoni M. 1999. SR141716A induces in rats a behavioral pattern opposite to that of CB1 receptor agonists. Zhongguo Yao Li Xue Bao 20:1103–1108.

Costa B, Colleoni M, Conti S, Trovato AE, Bianchi M, Sotgui ML, Giagnoni G. 2004. Repeated treatment with the synthetic cannabinoid WIN 55,212-2 reduces both hyperalgesia and production of pronociceptive mediators in a rat model of neuropathic pain. Br J Pharmacol 141:4–8.

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci USA 98:9371–9376.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384:83–87.

Cravatt BF, Lichtman AH. 2002. The enzymatic inactivation of the fatty acid amide class of signaling lipids. Chem Phys Lipids 121:135–148.

Cravatt BF, Prospero-Garcia O, Siuzdak G, Gilula NB, Henriksen SJ, Boger DL, Lerner RA. 1995. Chemical characterization of a family of brain lipids that induce sleep. Science 268:1506–1509.

Devane WA, Hanus L, Breur A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949.

Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Martin BR. 1999. Cannabimimetic fatty acid derivatives: the anandamide family and other endocannabinoids. Curr Med Chem 6:721–744.

Di Marzo V, De Petrocellis L, Fezza F, Ligresti A, Bisogno T. 2002. Anandamide receptors. Prostaglandins Leukot Essent Fatty Acids 66:377–391.

Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, et al. 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410:822–825.

Dinh TP, Freund TF, Piomelli D. 2002. A role for monoacylglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 2002:149–158.

Dogrul A, Gardell LR, Ma S, Ossipov MH, Porreca F, Lai J. 2002. "Knock-down" of spinal CB1 receptors produces abnormal pain and elevates spinal dynorphin content in mice. Pain 100:203–209.

Drew LJ, Harris J, Millns PJ, Kendall DA, Chapman V. 2000. Activation of spinal cannabinoid I receptors inhibits C-fibre driven hyperexcitable neuronal responses and increases [35]SGTPgammaS bindiing in the dorsal horn of the spinal cord of noninflamed and inflamed rats. Eur J Neurosci 12:2079–2086.

Egertova M, Giang DK, Cravatt BF, Elphick MR. 1998. A new perspective on cannabinoid signalling: complimentary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc R Soc Lond B 265:2081–2085.

Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice AS, Elphick MR. 2000. Cannabinoid CB1 receptor expression in rat spinal cord. Mol Cell Neurosci 15:510–521.

Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, James I. 2001. The role of central and peripheral cannabinoid 1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 92: 91–100.

Fukushima N, Ishii I, Contos JJ, Weiner JA, Chun J. 2001. Lysophospholipid receptors. Annu Rev Pharmacol Toxicol 41:507–534.

Giuffrida A, Beltramo M Piomelli D. 2001. Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. J Pharmacol Exp Ther 298:7–14.

Glaser ST, Abumrad N, Fatade F, Kaczocha M, Studholme KM, Deutsch DG. 2003. Evidence against the presence of an anandamide transporter. Proc Natl Acad Sci USA 100:4269–4274.

Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S. 1998. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. FEBS Lett 422:69–73.

Goya P, Jagerovic N, Hernandez-Folgado L, Martin MI. 2003. Cannabinoids and neuropathic pain. Mini Rev Med Chem 3:765–772.

Hanus L, Breur A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R, Fride E. 1999. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 96: 14228–14233.

Herzberg U, Eliav E, Bennett GJ, Kopin IJ. 1997. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuorpathic pain. Neurosci Lett 221:157–160.

Hillard CJ, Jarrahian A. 2000. The movement of N-arachidonoylethanolamine (anandamide) across cellular membranes. Chem Phys Lipids 108:123–134.

Hohmann AG. 2002. Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological, and neuroanatomical perspectives. Chem Phys Lipids 31:173–190.

Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. 2004. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. J Pharmacol Exp Ther 308:446–453.

Hohmann AG, Martin WJ, Tsou K, Walker JM. 1995. Inhibition of noxious stimulus-evoked activity of spinal cord dorsal horn neurons by the cannabinoid WIN 55,212-2. Life Sci 56:2111–2118.

Hohmann AG, Tsou K, Walker JM. 1998. Cannabinoid modulaton of wide dynamic range neurons in the lumbar dorsal horn of the rat by spinally administered WIN55,212-2. Neurosci Lett 257:119–122.

Ibrahim MM, Deng H, Zvonok AM, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, et al. 2003. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci USA 100:10529–10533.

Iwamura H, Suzuki H, Ueda Y, Kaya T, Inaba T. 2001. In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for the cannabinoid CB2 receptor. J Pharmacol Exp Ther 296:420–425.

Jaggar SI, Hasnie FS, Sellaturay S, Rice AS. 1998. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. Pain 76:189–199.

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, et al. 2003. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 9:76–81.

Kelly S, Chapman V. 2001. Selective cannabinoid CB1 receptor activation inhibits spinal nociceptive transmission in vivo. J Neurophys 86:3061–3064.

Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI. 1997. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur J Pharmacol 331:R1–R2.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, et al. 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283:401–404.

Leung D, Hardouin C, Boger DL, Cravatt BF. 2003. Discovering potent and selective inhibitors of enzymes in complex proteomes. Nat Biotechnol 21:687–691.

Lichtman AH, Cook SA, Martin BR. 1996. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 276:585–593.

Lichtman AH, Hawkins EG, Griffin G, Cravatt BF. 2002. Pharmacological activity of fatty acid amides is regu-

lated, but not mediated, by fatty acid amide hydrolase in vivo. J Pharmacol Exp Ther 302:73–79.

Lichtman AH, Leung D, Shelton C, Saghatelian A, Hardouin C, Boger DL, Cravatt BF. 2004a. Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. J Pharmacol Exp Ther, to appear.

Lichtman AH, Martin BR. 1991. Spinal and supraspinal mechanisms of cannabinoid-induced antinociception. J Pharmacol Exp Ther 258:517–523.

Lichtman AH, Martin BR. 1997. The selective cannabinoid antagonist, SR141716A, blocks cannabinoid-induced antinociception following intraventricular administration in rats. Brain Res 629:300–304.

Lichtman AH, Shelton CC, Advani T, Cravatt BF. 2004b. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 109:319–327.

Lichtman AH, Smith PB, Martin BR. 1992. The antinociceptive effects of intrathecally administered cannabinoids are influenced by lipophilicity. Pain 51:19–26.

Lim G, Sung B, Ji RR, Mao J. 2003. Upregulation of spinal cannabinoid-1-receptors following nerve injury enhances the effects of Win 55,212-2 on neuropathic pain behavior in rats. Pain 105:275–283.

Maccarrone M, Bari M, Battista N, Finazzi-Agro A. 2002. Endocannabinoid degradation, endotoxic shock, and inflammation. Curr Drug Targets Inflamm Allergy 1: 53–63.

Malan TP, Ibrahim MM, Deng H, Liu H, Mata HP, Vanderah TW, Porreca F, Makriyannis A. 2001. CB2 cannabinoid receptor-mediated peripheral antinociception. Pain 93:239–245.

Malan TP, Ibrahim MM, Vanderah TW, Makriyannis A. Porreca F. 2002. Inhibition of pain responses by activation fo CB2 cannabinoid receptors. Chem Phys Lipids 121:191–200.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B. 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534.

Martin BR, Lichtman AH. 1998. Cannabinoid transmission and pain perception. Neurobiol Dis 5:447–461.

Martin WJ, Hohmann AG, Walker JM. 1996. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptiv effects. J Neurosci 16:6601–6611.

Martin WJ, Lai NK, Patrick SL, Tsou K. Walker JM. 1993. Antinociceptive actions of cannabinoids following intraventricular administration in rats. Brain Res 629:300–304.

Martin WJ, Loo CM, Basbaum AI. 1999. Spinal cannabinoids are anti-allodynic in rats with persistent inflammation. Pain 82:199–205.

Martin WJ, Patrick SL, Coffin PO, Tsou K, Walker JM. 1995. An examination of central sites of action of cannabinoid-induced antinociception in the rat. Life Sci 56: 2103–2109.

Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, Ferri G-L, Sibaev A, Storr M, Lutz B. 2004. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 113:1202–1209.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564.

Mechoulam R. 1986. The pharmacohistory of Cannabis sativa. Boca Raton, FL: CRS Press.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds cannabinoid receptors. Biochem Pharmacol 50:83–90.

Mechoulam R, Fride E, Hanus L, Sheskin T, Bisogno T, Di Marzo V, Bayewitch M, Vogel Z. 1997. Anandamide may mediate sleep induction. Nature 389:25–26.

Meng ID, Manning BH, Martin WJ, Fields HL. 1998. An analgesia circuit activated by cannabinoids. Nature 395: 381–383.

Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, et al. M. 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80: 67–82.

Monhemius R, Azami J, Green DL, Roberts MH. 2001. CB1 receptor mediated analgesia from the nucleus reticularis gigantocellularis pars alpha is activated in an anmial model of neuropathic pain. Brain Res 908:67–74.

Munro S, Thomas KL, Abu-Shaar M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61–65.

Nackley AG, Makriyannis A, Hohmann AG. 2003. Selective activation of cannabinoid CB2 receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 119:747–757.

Nilius B, Vriens J, Prenen J, Droogmans G, Voets T. 2004. TRPV4 calcium entry channel: a paradigm for gating diversity. Am J Physiol Cell Physiol 286:C195–205.

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279: 5298–5305.

Patricelli, MP, Cravatt, BF. 2001. Proteins regulating the biosynthesis and inactivation of neuromodulatory fatty acid amides. Vitam Horm 62:95–131.

Pertwee RG. 2001. Cannabinoid receptors and pain. Prog Neurobiol 63:569–611.

Portier M, Rinaldi-Carmona M, Pecceu F, Combes T, Poinot-Chazel C, Calandra B, Barth F, le Fur G, Casellas P. 1999. SR 1444528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. J Pharmacol Exp Ther 288:582–589.

Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, Malan TP. 2003. Inhibition of

inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. Anesthesiology 99:955–960.

Rice AS. 2001. Cannabinoids and pain. Curr Opin Investig Drugs 2:399–414.

Richardson JD, Aanonsen L, Hargreaves K. 1998a. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. J Neurosci 18:451–457.

Richardson JD, Kilo S, Hargreaves KM. 1998b. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 75:111–119.

Rinaldi-Carmona M, et al. 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240–244.

Rodriguez de Fonseca F, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, Murillo-Rodriguez E, Giuffrida A, LoVerme J, Gaetani S, et al. 2001. An anorexic lipid mediator reguated by feeding. Nature 414:209–212.

Sanudo-Pena MC, Strangman NM, Mackie K, Walker JM, Tsou K. 1999. CB1 receptor localization in rat spinal cord and roots, dorsal root ganglion, and peripheral nerve. Zhongguo Yao Li Xue Bao 20:1115–1120.

Shire D, Calandra B, Bouaboula M, Barth F, Rinaldi-Carmona M, Casellas P, Ferrara P. 1999. Cannabinoid receptor interactions with the antagonists SR 141716A and SR 144528. Life Sci 65:627–635.

Siegling A, Hofmann HA, Denzer D, Mauler F, Vry D. 2001. Cannabinoid CB1 receptor upregulation in a rat model of chronic neuropathic pain. Eur J Pharmacol 415:R5–R7.

Sit S-Y, Xie K. 2002. Bisarylimidazolyl fatty acid amide hydrolase inhibitors. Patent W0 02/087569.

Sit S-Y, Xie K. 2003. (Oxime)carbamoyl fatty acid amide hydrolase inhibitors. Patent Application US 2003/0195226 A1.

Smith FL, Fujimori K, Lowe J, Welch SP. 1998. Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. Pharmacol Biochem Behav 60:183–191.

Smith PB, Martin BR. 1992. Spinal and supraspinal mechanisms of cannabinoid-induced antinociception. J Pharmacol Exp Ther 258:517–523.

Stella N, Schweitzer P, Piomelli D, 1997. A second endogenous cannabinoid that modulates long term potentiation. Nature 388:773–778.

Strangman NM, Patrick SL, Hohmann AG, Tsou K, Walker JM. 1998. Evidence for a role for endogenous cannabinoids in the modulation of acute and tonic pain sensitivity. Brain Res 813:323–328.

Strangman NM, Walker JM, 1999. The cannabinoid WIN 55,212-2 inhibits the activity dependent facilitation of spinal nociceptive responses. J Neurophys 81:472–477.

Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, Yamshita A, Waku K, 1996. Transacylase-mediated and phosphodiesterase-mediated synthesis of N-arachidonylethanolamine, an endogenous cannabinoid receptor ligand in rat brain microsomes. Comparison with synthesis from free arachidonic acid and ethanolamine. Eur J Biochem 240:53–62.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM, 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Valverde O, Ledent C, Beslot F, Parmentier M, Roques BP, 2000. Reduction of stress-induced analgesia but not exogenous opioid effects in mice lacking CB1 receptors. Eur J Neurosci 12:533–539.

Varvel SA, Lichtman AH. 2002. Evaluation of CB1 receptor knockout mice in the Morris water maze. J Pharmacol Exp Ther 301:915–924.

Walker JM, Huang SM. 2002. Cannabinoid analgesia. Pharmacol Ther 95:127–135.

Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC. 1999. Pain modulation by release of the endogenous cannabinoid anandamide. Proc Natl Acad Sci USA 96:12198–12203.

Yaksh TL. 1981. The antinociceptive effects of intrathecally-administered levonantradol and deacetyllevonantradol in the rat. J Clin Pharmacol 21:334S–340S.

Zhang J, Hoffet C, Vu HK, Groblewski T, Ahmad S, O'Donnell D. 2003. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur J Neurosci 17:2750–2754.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. 1999. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci 96:5780–5785.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED. 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457.