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Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Inhibitors Produce Anti-Allodynic Effects in Mice through Distinct Cannabinoid Receptor Mechanisms

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Abstract

The endocannabinoids, anandamide and 2-arachidonoylglycerol, are predominantly regulated by the respective catabolic enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Inhibition of these enzymes elevates endocannabinoid levels and attenuates neuropathic pain. In the present study, CB₁ and CB₂ receptor deficient mice were subjected to chronic constriction injury (CCI) of the sciatic nerve to examine the relative contribution of each receptor for the anti-allodynic effects of the FAAH inhibitor, PF-3845, and the MAGL inhibitor, JZL184. CCI caused marked hypersensitivity to mechanical and cold stimuli, which was not altered by deletion of either the CB₁ or CB₂ receptor, but was attenuated by gabapentin, as well as by each enzyme inhibitor. Whereas PF-3845 lacked anti-allodynic efficacy in both knockout lines, JZL184 did not produce anti-allodynic effects in CB₁ (–/–) mice, but retained its anti-allodynic effects in CB₂ (–/–) mice. These data indicate that FAAH and MAGL inhibitors reduce nerve-injury related hyperalgesic states through distinct cannabinoid receptor mechanisms of action. In conclusion, although endogenous cannabinoids do not appear to play a tonic role in long-term expression of neuropathic pain states, both FAAH and MAGL represent potential therapeutic targets for the development of pharmacological agents to treat chronic pain resulting from nerve injury.

Keywords

endogenous cannabinoid; fatty acid amide hydrolase (FAAH); monoacylglycerol lipase (MAGL); neuropathic pain; anandamide; 2-arachidonoylglycerol (2-AG)

Introduction

Chronic pain decreases quality of life and also places a substantial economic burden at the level of the individual, through medical spending, analgesics, and loss of paid work hours,

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as well as society at large, through state medical expenses and lost productivity³³. Neuropathic pain results from injury to peripheral nerves and is a particularly intractable form of chronic pain because of its resistance to currently available analgesics. *Cannabis sativa*, as well as its extracts, has been used for thousands of years to treat various types of pain¹⁵. However, the primary psychoactive component of *Cannabis sativa*, Δ^9 -tetrahydrocannabinol¹², also elicits undesirable psychomimetic side effects, limiting its potential therapeutic efficacy. Thus, recent attention has turned to the endogenous cannabinoid (i.e., endocannabinoid) system, in search of potential targets for novel analgesics^{30,32}.

The endocannabinoid system is comprised of two cloned cannabinoid receptors, CB₁ and CB₂^{23,26}; the endogenous ligands for these receptors, including anandamide (AEA)¹⁰ and 2-arachidonoyl glycerol (2-AG)²⁴; and enzymes responsible for the biosynthesis and degradation of the endocannabinoids. Anandamide is hydrolyzed by fatty acid amide hydrolase (FAAH)⁷, and 2-AG is primarily metabolized by monoacylglycerol lipase (MAGL)^{3,11}. Whereas exogenously administered endocannabinoids are rapidly degraded by FAAH and MAGL, pharmacological inhibition of these enzymes results in elevated endocannabinoids in brain and spinal cord tissue^{7,14,19}. Thus, inhibiting FAAH and MAGL leads to increased bioavailability of AEA and 2-AG, respectively.

Cannabinoids modulate different types of pain through CB₁ and/or CB₂ receptor mediated mechanisms of action. A commonly used murine neuropathic pain model, chronic constriction injury (CCI) of the sciatic nerve, causes hypersensitivity to radiant heat stimuli (i.e., hyperalgesia)^{18,22}, as well as nociceptive responses to typically non-nociceptive stimuli (i.e., allodynia) including touch⁵ and cold³⁷. CCI-induced mechanical allodynia and thermal hyperalgesia were reduced by WIN 55,212-2, a mixed CB₁/CB₂ receptor agonist. Pretreatment with either the CB₁ receptor selective antagonist, rimonabant, or the CB₂ receptor selective antagonist, SR144528 prevented these effects of WIN 55,212-2, indicating that each cannabinoid receptor subtype is necessary for the anti-allodynic effects of WIN 55,212-2¹⁶.

Administration of inhibitors of endocannabinoid catabolic enzymes also attenuates neuropathic pain. For example, repeated administration of the irreversible FAAH inhibitor URB597 attenuated CCI-induced mechanical allodynia and thermal hyperalgesia³¹. These effects were completely blocked by pretreatment with either CB₁ or CB₂ receptor antagonists. Similarly, the α -keto-heterocycle reversible FAAH inhibitor, OL-135, attenuated mechanical allodynia in the partial ligation spinal nerves rat model, but this effect was blocked by pretreatment with SR144528 or naloxone⁴. In contrast, OL-135 attenuated CCI-induced mechanical and cold allodynia in mice, and these effects were completely blocked by pretreatment with either CB₁ or CB₂ receptor antagonists, but not by naltrexone or a TRPV1 receptor antagonist¹⁴. Inhibition of MAGL with JZL184 also attenuated CCI-induced mechanical and cold allodynia in mice¹⁴. As with the FAAH inhibitors, the anti-allodynic effects of JZL184 were completely blocked by rimonabant, but not by SR144528, indicating that distinct cannabinoid receptor mechanisms of action underlie the anti-allodynic effects of FAAH and MAGL inhibitors¹⁴.

The present study was designed to address the seemingly disparate cannabinoid receptor mechanisms through which FAAH and MAGL inhibition reduces neuropathic pain. A recently developed FAAH inhibitor, PF-3845, offers many advantages over previous FAAH inhibitors, including increased FAAH selectivity and a duration of activity *in vivo* of at least 24 hours². This increased temporal window of activity allowed us to use the same pretreatment times for both inhibitors. The GABA analog gabapentin was also assessed, as a positive control. Whereas previous studies used pharmacological approaches to discern

cannabinoid mechanism of action, the present study employed CB₁ and CB₂ receptor deficient mice to ask the following questions: First, does genetic deletion of CB₁ or CB₂ attenuate or exacerbate CCI-induced nociception? Second, are the anti-allodynic effects of FAAH inhibition prevented in CB₁ or CB₂ receptor deficient mice? Third, what is the contribution of CB₁ and CB₂ receptors for the anti-allodynic effects caused by MAGL inhibition?

Materials and Methods

Animals

Subjects consisted of male and female CB₁ (-/-) and CB₂ (-/-) mice as well as their respective littermate controls, CB₁ (+/+) and CB₂ (+/+) mice from the Center Transgenic Colony at Virginia Commonwealth University. CB₁ (-/-) and CB₂ (-/-) mice were backcrossed onto a C57BL/6J background for 13 and 6 generations, respectively. The subjects were housed in a temperature (20-22 °C) and humidity controlled, AAALAC-approved facility, with *ad libitum* access to food and water. Mice weighed approximately 25 g, and were housed 4-6 per cage and maintained on a 12:12 light cycle. Based on previous studies from our laboratory^{14,19}, the sample size for each treatment group was 6-11 mice/group. All experiments were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. After testing was complete, all mice were humanely euthanized via CO₂ asphyxia, followed by rapid cervical dislocation.

Chronic constriction injury (CCI)

Mice were administered acetaminophen (2.4 mg/ml in drinking water) from 24 h before surgery until 48 h post surgery. Anesthesia was maintained throughout surgery using constant inhalation of 1.5% isoflurane. The right hind leg was shaved and the area swabbed with Betadine solution, then ethanol. An incision was made in the skin posterior to the femur, and the sciatic nerve was visualized and isolated following separation of the muscle. The nerve was ligated twice 5-0 (1.0 metric) black silk braided suture (Surgical Specialties Corporation, Reading, PA). The surrounding muscle and skin were then sutured with 6-0 nylon. Mice were recovered in a heated cage and observed for approximately 2 h before being returned to the vivarium.

Behavior testing

Allodynia was initially tested two months after surgery. Previous reports from our lab and others indicate that CCI-induced hyperalgesia and allodynia persist for months after injury^{18,22}, so this time point was chosen to reflect a developed, chronic pain state. Mice were brought into the testing room, weighed, and allowed to acclimate for at least 1 h before the start of each experiment. The mice were placed inside ventilated polycarbonate chambers on an aluminum mesh table and allowed to acclimate to the apparatus for 60 min prior to testing. For the gabapentin experiment, mice were immediately placed in the apparatus after injection, and tested 60 min later. In the experiments using PF-3845 or JZL184, mice were injected, returned to their home cage, and then placed in the test apparatus 60 min prior to testing (total absorption time was 120 min)¹⁴.

von Frey Test—Mechanical allodynia was assessed with von Frey filaments (North Coast Medical, Morgan Hill, CA), using the “up-down” method⁵. The plantar surface of each hind paw was stimulated 5 times with each filament (0.16 – 6.0 g), at a frequency of roughly 2 Hz, starting with the 0.6 g filament and increasing weight. Responses were scored when the mouse clutched or lifted its paw. Paw lifting in response to three or more stimulations was coded as a positive response. Once a positive response was detected, sequentially lower weight filaments were used to assess paw withdrawal threshold.

Acetone-induced Cold Allodynia Test—Approximately 30 min after completing the von Frey test, 10 μ l of acetone (99% HPLC grade, Fisher Bioscience) was projected via air burst, using a 200 μ l pipette (Rainin Instruments, Oakland, CA) onto the plantar surface of each hind paw ^{6,9}. Total time lifting or clutching each paw was recorded, with a maximum cutoff time of 20 s ⁹.

Drugs

Gabapentin was purchased from Cayman Chemical (Ann Arbor, MI). JZL184 ¹⁹ and PF-3845 ² were synthesized as described previously. All drugs were dissolved in a vehicle consisting of ethanol, Alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and saline in a ratio of 1:1:18, and were injected intraperitoneally (i.p.) at a volume of 10 μ l/g body mass. All solutions were warmed to room temperature prior to injection. Single doses of 50 mg/kg gabapentin, 10 mg/kg PF-3845, or 40 mg/kg JZL184, were chosen based on the literature and previous data from our lab ^{2,14,20}. A within-subjects design was used to reduce the total number of animals needed for this study. Mice were randomly assigned to drug treatment group by a random number generator, and treatment conditions were counterbalanced across test days. In order to avoid carry-over effects, drug washout time between treatments was at least two days for gabapentin, four days for PF-3845, and seven days for JZL184. In order to minimize potential bias, the experimenter was blinded to genotype and drug treatment.

Data Analysis

All data are reported as mean $\pm$ SEM and were analyzed using two-way mixed factorial analysis of variance (ANOVA), with genotype as the between subjects measure, and either paw or drug as the within subjects measure. For the drug studies, data from knockout mice and their respective wildtype littermates were grouped and analyzed by respective genotype (i.e., CB₁ (+/+) vs. CB₁ (-/-) or CB₂ (+/+) vs. CB₂ (-/-)). Follow-up comparisons were made using the Bonferroni test. All animals were included in analyses. Differences between groups were considered statistically significant at $p < 0.05$.

Results

Genetic deletion of CB₁ or CB₂ does not affect long-term expression of allodynia in the CCI model

Across genotypes, CCI caused a significant decrease in paw withdrawal threshold in response to von Frey filament stimulation (i.e., mechanical allodynia) [$F(3,68) = 57$; $p < 0.0001$; Figure 1A] and acetone-induced cold allodynia [$F(3,68) = 49$; $p < 0.0001$; Figure 1B]. There was no effect of genotype. Genetic deletion of the CB₁ receptor did not affect the magnitude of mechanical ($p = 0.90$; Figure 1A) or cold allodynia ($p = 0.56$; Figure 1B). Similarly, genetic deletion of the CB₂ receptor had no effect on the magnitude of mechanical ($p = 0.51$; Figure 1A) or cold allodynia ($p = 0.99$; Figure 1B).

The anti-allodynic effects of gabapentin are cannabinoid receptor independent

The GABA analogue, gabapentin (50 mg/kg i.p.), was used as a positive control, in order to examine whether CB₁ (-/-) or CB₂ (-/-) mice would show altered anti-allodynic effects to noncannabinoid agents. In the CB₁ genotypes, there was a main effect of gabapentin treatment on mechanical allodynia [$F(1,15) = 44$; $p < 0.0001$; Figure 2A] and cold allodynia [$F(1,15) = 94$; $p < 0.0001$; Figure 2B], with no differences between genotypes for either measure [$p = 0.32$ for CB₁ (+/+) and 0.49 for CB₁ (-/-)]. There was no significant interaction between gabapentin and genotype on mechanical ($p = 0.28$) or cold allodynia ($p = 0.50$) in the CB₁ genotypes. Similarly, in the CB₂ genotypes, gabapentin significantly attenuated mechanical allodynia [$F(1,18) = 62$; $p < 0.0001$; Figure 2C] and cold allodynia

[F(1,18) = 58; $p < 0.0001$; Figure 2D], with no genotype differences for either measure ($p = 0.70$ and 0.98 , respectively). There was no significant interaction between gabapentin and genotype on mechanical ($p = 0.69$) or cold allodynia ($p = 0.46$). Gabapentin had no effect on either measure in the non-injured (i.e., contralateral paw; Table1).

The anti-allodynic effects of FAAH inhibition require CB₁ and CB₂ receptors

Inhibition of fatty acid amide hydrolase (FAAH) has been shown to attenuate CCI-induced allodynia¹⁴. In the CB₁ genotypes, there was a main effect of the FAAH inhibitor PF-3845 (10 mg/kg, i.p.) on CCI-induced mechanical allodynia [F(1,16) = 5.05; $p < 0.05$; Figure 3A] and cold allodynia [F(1,16) = 9.11; $p < 0.01$; Figure 3B]. Although there was no significant interaction between PF-3845 and genotype on mechanical ($p = 0.09$) or cold allodynia ($p = 0.10$), planned comparisons revealed that PF-3845 had anti-allodynic effects in CB₁ (+/+) mice ($p < 0.05$ and 0.01 , respectively), whereas CB₁ (-/-) mice were completely resistant to the effects of PF-3845 on mechanical ($p = 0.57$) and cold allodynia ($p = 0.26$). In the CB₂ mice, there was no significant interaction between the FAAH inhibitor PF-3845 and genotype on mechanical allodynia ($p = 0.07$), although there was a significant interaction between genotype and cold allodynia [F(1,18) = 6.40; $p < 0.05$]. Follow up comparisons revealed that PF-3845 significantly reduced mechanical ($p < 0.05$; Figure 3C) and cold allodynia ($p < 0.05$, Figure 3D) in CB₂ (+/+) mice, whereas CB₂ (-/-) mice were completely resistant to the effects of PF-3845 on mechanical ($p = 0.99$) cold ($p = 0.33$) allodynia. FAAH inhibition with PF-3845 had no effect on either measure in the contralateral paw (Table1). These data indicate that FAAH inhibition attenuates allodynia via a mechanism that requires activation of both CB₁ and CB₂ receptor subtypes.

The anti-allodynic effects of MAGL inhibition require CB₁ and not CB₂ receptors

JZL184 irreversibly inhibits MAGL, and attenuates CCI-induced allodynia¹⁴. In the CB₁ genotypes, there was a main effect of the MAGL inhibitor JZL184 (40 mg/kg, i.p.) on mechanical [F(1,15) = 5.03; $p < 0.05$; Figure 4A] and cold allodynia [F(1,15) = 9.48; $p < 0.01$; Figure 4B]. There was no significant interaction between JZL184 and genotype on mechanical ($p = 0.10$) or cold allodynia ($p = 0.23$), however planned comparisons revealed that, in CB₁ (+/+) mice, JZL184 attenuated mechanical and cold allodynia ($p < 0.05$ and 0.01 , respectively), whereas in CB₁ (-/-) mice, JZL184 had no effect on mechanical ($p = 0.66$) or cold allodynia ($p = 0.32$). In other words, the anti-allodynic effects of JZL184 were blocked by genetic deletion of the CB₁ receptor. In the CB₂ genotypes, there was a main effect of JZL184 on mechanical [F(1,22) = 8.47; $p < 0.01$; Figure 4C] and cold allodynia [F(1,22) = 22.9; $p < 0.0001$; Figure 4D]. There was no significant interaction between JZL184 and genotype on mechanical ($p = 0.58$) or cold allodynia ($p = 0.34$). In other words, JZL184 had anti-allodynic effects, regardless of the presence or absence of the CB₂ receptor. Similarly, in the CB₂ genotypes, JZL184 significantly attenuated mechanical ($p < 0.01$; 0.05 , respectively) and cold allodynia ($p < 0.001$; 0.05 , respectively). JZL184 had no effect on either measure in the contralateral paw (Table1). These data indicate that CB₁ receptors, but not CB₂ receptors, are necessary for the anti-allodynic effects caused by MAGL inhibition.

Discussion

In the present study, we tested the hypothesis that inhibiting endocannabinoid catabolic enzymes would attenuate nociception following chronic nerve injury. To address this question, we used the FAAH inhibitor, PF-3845, and the MAGL inhibitor, JZL184. PF-3845 offers advantages over other FAAH inhibitors, in that it is highly selective for FAAH and also has a long duration of activity². As we previously reported¹⁴, the FAAH inhibitor, PF-3845, as well as the MAGL inhibitor, JZL184, attenuated chronic constriction injury

(CCI) induced mechanical and cold allodynia in wildtype mice, but did not affect responses in the non-injured paw. The present study extended the results of our previous findings by using a complementary genetic approach to examine cannabinoid receptor mechanism of action. FAAH inhibition did not elicit anti-allodynic effects in CB₁ (-/-) or CB₂ (-/-) mice, indicating that both receptor subtypes are necessary for the expression of these effects. On the other hand, the anti-allodynic effects of MAGL inhibition were prevented in CB₁ (-/-), but not in CB₂ (-/-) mice, indicating that these actions of 2-AG are driven by a CB₁ selective mechanism of action. Thus, it appears that anandamide and 2-AG elicit anti-allodynic effects through distinct cannabinoid receptor mechanisms of actions. Elevating endogenous levels of anandamide produces anti-allodynic effects through the activation of both CB₁ and CB₂ receptors. In contrast, these data suggest that CB₁ receptors are necessary for the anti-allodynic effects resulting from elevated levels of 2-AG, but CB₂ receptors are dispensable.

Inhibition of MAGL, via JZL184 results in a 10-fold increase in brain levels of 2-AG and has antinociceptive effects¹⁹. Unlike FAAH inhibition, MAGL inhibition via JZL184 causes a subset of THC-like effects in vivo including hypothermia, hyperreflexia, and hypomotility¹⁹. In the CCI model, JZL184 attenuates allodynia via a FAAH-independent mechanism of action and is reversed by the CB₁ receptor selective antagonist, rimonabant¹⁴. However, rimonabant is also an inverse agonist of CB₁¹⁷, which may have contributed to this effect. The present data demonstrate that, similar to rimonabant pretreatment¹⁴, genetic deletion of CB₁ also prevents the anti-allodynic effects of MAGL inhibition, and indicate that the previous findings were not based on inverse agonist effects of rimonabant. In the present study, CB₂ receptor deletion did not affect the efficacy of JZL184 to attenuate CCI-induced allodynia, which is consistent with our previous finding using the CB₂ selective antagonist, SR144528¹⁴. The future development of MAGL (-/-) mice would provide a powerful complementary tool for understanding 2-AG modulation of neuropathic pain.

The endocannabinoids anandamide and 2-AG bind to both CB₁ and CB₂ receptors, yet these data indicate that FAAH inhibition appears to reduce neuropathic pain via a mechanism that requires both receptor subtypes, whereas MAGL inhibition works by a mechanism that requires CB₁, but not CB₂ receptor activation. Both CB₁ and CB₂ have been shown previously to be involved in the antihyperalgesic and anti-allodynic effects of FAAH inhibition following nerve injury^{14,16,30-31}. The CB₁ receptor is expressed in nervous tissues and may ameliorate neuropathic pain by inhibiting nociception in central pain pathways or on peripheral nociceptors¹. CB₂ receptors are expressed peripherally and also centrally on microglia⁴⁰ and possibly also neural tissue³⁵. Rats subjected to CCI expressed higher levels of anandamide and 2-AG in the periaqueductal gray and rostral ventromedial medulla²⁸, where endocannabinoids may play a role in pain reduction³⁸. In the dorsal raphe²⁷, CCI increases levels of AEA, but not 2-AG, indicating that region-specific alterations in endogenous cannabinoids may be having different effects on pain modulation following nerve injury.

In addition to both cannabinoid receptors, anandamide also binds to the transient receptor potential vanilloid (TRPV1) receptor³⁴. Thus, the TRPV1 receptor may contribute to the effects of anandamide on nociception, resulting from FAAH inhibition. However, previous research demonstrated that the TRPV1 antagonist, capsazepine did not attenuate the anti-allodynic effects of FAAH inhibition, via URB597 or OL-135, in mice subjected to CCI¹⁴. Although a possible alternative approach would be to compare the anti-allodynic effects of FAAH and/or MAGL inhibition in TRPV1 (-/-) mice, these mice show a marked baseline analgesic phenotype compared to wildtype littermates³⁶, thus confounding interpretations of their use in the present model.

Genetic deletion of either cannabinoid receptor subtype did not affect the expression of CCI-induced allodynia, indicating a lack of endocannabinoid tone following long-term injury to the sciatic nerve. Moreover, the observation that gabapentin retained its anti-allodynic efficacy in cannabinoid receptor deficient mice indicates no alterations in responsivity to noncannabinoid compounds. It has been previously reported that transgenic mice overexpressing CB₂ display CCI-induced hyperalgesia and mechanical allodynia not only in the paw ipsilateral to nerve injury, but also in the contralateral paw²⁹. We did not observe any such genotype differences in mechanical or cold allodynia in the contralateral paw (Table 1). These differences in findings are likely due to subtle variations in methodology, such as differences in testing apparatus and postsurgical recovery time. For example, although both labs used similar calibrated nylon filaments for the von Frey test, Racz and colleagues also used a Dynamic Aesthesiometer, and whereas we used the acetone-induced cold allodynia test, they used the cold plate test. In the present study, mice were examined weeks after the initial development of CCI-induced allodynia, not during the initial stages of neuropathic pain development. It was recently reported that the synthetic CB₂ agonist NESS400 given early after nerve injury reduced allodynia and proinflammatory mediators in dorsal horn extracts following CCI²¹. Given these differences, however, we observed similar effects CCI-induced allodynia in the paw ipsilateral to the nerve injury. The role of MAGL inhibition in the early development of CCI-induced neuropathic pain is currently unknown and may be CB₂ receptor dependent.

It has been previously reported that PF-3845 increases AEA in whole mouse brain², and another FAAH inhibitor, URB597, increased AEA levels in whole brain as well as in spinal cord of mice¹⁴. Similarly, JZL184 significantly increased 2-AG levels in whole mouse brain and spinal cord¹⁴. Indeed, data using the rat spinal nerve ligation (SNL) model indicate that both AEA and 2-AG levels are increased in dorsal root ganglia (DRG) proximal and ipsilateral to nerve ligation, as compared with DRG contralateral to injury, sham, or naïve rats²⁵. This localized increase in endocannabinoids may be driven by the inflammatory response to nerve insult. A recent report by Guasti and colleagues¹³ indicated that chronic inhibition of microglial activation with minocycline attenuated SNL-induced increases in 2-AG but had no effect on AEA in lumbar spinal cord ipsilateral to SNL, as compared with tissue contralateral to nerve injury. Although CCI had no effect on AEA or 2-AG levels in whole brain or spinal cord in mice¹⁴, these data from rat studies suggest that regional differences in pools of these endocannabinoids may, at least in part, mediate the observed anti-allodynic effects of FAAH or MAGL inhibition in the present study.

In the present study, acute administration of the enzyme inhibitors PF-3845 and JZL184 was less efficacious at attenuating CCI-induced allodynia than gabapentin. These data are in agreement with our previous report, in which the reversible FAAH inhibitor, OL-135, was less effective at blocking allodynia than gabapentin, when tested two weeks after CCI surgery¹⁴. It has been reported that repeated administration of URB597 has increased anti-allodynic efficacy in the murine CCI model³¹. Thus, we are currently examining whether repeated administration of PF-3845 or JZL184 results in increased anti-allodynic effects, as compared with acute administration or either enzyme inhibitor.

Pharmacological inhibition of FAAH increases anandamide levels in whole brain and spinal cord and results in antinociception^{2,8,14}. Similarly, FAAH deficient knockout mice express 15-fold higher levels of anandamide in whole brain, as compared with wildtype littermates⁸. FAAH(−/−) mice display an antinociceptive phenotype in a variety of tests, including tail immersion, hotplate analgesia, carrageenan-induced paw edema and hyperalgesia, and formalin-induced paw guarding^{18,39}. However, in the CCI model, no observed phenotypic difference have been reported between FAAH(−/−) and FAAH(+/+) mice in thermal hyperalgesia or allodynia^{14,18}, suggesting that FAAH deletion over the course of the

development of chronic pain affects resultant pain outcomes differently than acute FAAH inhibition.

In conclusion, the results of the present study indicate that pharmacological inhibition of FAAH or MAGL ameliorates sensitized nociceptive responses that persist for over two months in mice subjected to chronic constriction injury of the sciatic nerve. Whereas the CB₁ and CB₂ receptors are necessary for the anti-allodynic effects of FAAH inhibitors, only CB₁ receptors are necessary for the anti-allodynic effects caused by MAGL inhibition. The specific mechanisms through which endocannabinoids act upon CB₁ and CB₂ to differentially reduce allodynia are currently under investigation. Given this evidence of their involvement in neuropathic pain, FAAH and MAGL offer potential targets for new therapeutic analgesics.

Perspective

This article presents data addressing the cannabinoid receptor mechanisms underlying the anti-allodynic actions of endocannabinoid catabolic enzyme inhibitors in the mouse sciatic nerve ligation model. Fatty acid amide hydrolase and monoacylglycerol lipase inhibitors reduced allodynia through distinct cannabinoid receptor mechanisms. These enzymes offer potential targets to treat neuropathic pain.

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Figure 1.

Chronic constriction injury (CCI) elicited mechanical and cold allodynia. Mice were tested for mechanical allodynia using von Frey filaments (**Panel A**) and acetone-induced cold allodynia (**Panel B**). CCI caused a similar degree of nociception in the paw ipsilateral to the nerve injury, regardless of genotype. Open bars, control paw; filled bars, CCI paw. Data expressed as mean $\pm$ SEM. (n = 7-11). **p < 0.01 vs. contralateral paw.

Figure 2.

Gabapentin reversed chronic constriction injury (CCI) induced mechanical and cold allodynia. Mice were subjected to CCI and tested for mechanical allodynia using von Frey filaments (**Panel A, C**) and acetone-induced cold allodynia (**Panel B, D**). Mice were treated with 50 mg/kg gabapentin (i.p.) 60 min before testing. Data represented are from ipsilateral paws. Control paws did not differ between drug treatments or genotypes. Open bars, vehicle; filled bars, gabapentin. Data expressed as mean $\pm$ SEM. (n = 6-11). ** p < 0.01, *** p < 0.001 vs. vehicle.

Figure 3.

The FAAH inhibitor, PF-3845, attenuated chronic constriction injury (CCI) induced mechanical and cold allodynia. Mice were subjected to CCI and tested for mechanical allodynia using von Frey filaments (**Panel A, C**) and acetone-induced cold allodynia (**Panel B, D**). Mice were treated with 10 mg/kg PF-3845 (i.p.) 120 min before testing. PF-3845 attenuated CCI-induced allodynia in wild type mice, whereas genetic deletion of either the CB₁ (**Panel A, B**) or the CB₂ (**Panel C, D**) receptor completely blocked the anti-allodynic effects of FAAH inhibition. Data from paws ipsilateral to nerve injury are shown. Control paws did not differ between drug treatments or genotypes. Open bars, vehicle; filled bars, PF-3845. Data expressed as mean $\pm$ SEM. (n = 7-11). * p < 0.05, ** p < 0.01 vs. vehicle; # p < 0.05, ## p < 0.01 vs. PF-3845 treated CB₁(+/+) or CB₂(+/+) mice, respectively.

Figure 4.

The MAGL inhibitor, JZL184, attenuated chronic constriction injury (CCI) induced mechanical and cold allodynia. Mice were subjected to CCI and tested for mechanical allodynia using von Frey filaments (**Panel A, C**) and acetone-induced cold allodynia (**Panel B, D**). Mice were treated with 40 mg/kg JZL184 (i.p.) 120 min before testing. JZL184 attenuated CCI-induced allodynia in wild type mice, whereas genetic deletion of the CB₁ (**Panel A, B**) receptor completely prevented this anti-allodynic effect. However, deletion of the CB₂ receptor (**Panel C, D**) did not affect the anti-allodynic effects of with JZL184. Data from ipsilateral paws shown. Control paws did not differ between drug treatments or genotypes. Open bars, vehicle; filled bars, JZL184. Data expressed as mean $\pm$ SEM. (n = 6-11). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. vehicle; ## p < 0.01 vs. JZL184 treated CB₁(+/+) mice.

Table 1

Genotype and drug treatment did not affect behavior in the hind paw contralateral to chronic constriction injury. Mechanical allodynia data reflect grams of pressure and cold allodynia data represent time (s) spent lifting or clutching the acetone-treated paw. Data expressed as mean $\pm$ SEM

Mechanical allodynia				
Genotype	Vehicle	Gabapentin	PF-3845	JZL184
CB ₁ (+/+)	4.73 $\pm$ 0.41	4.59 $\pm$ 0.33	2.87 $\pm$ 0.38	2.67 $\pm$ 0.46
CB ₁ (-/-)	4.33 $\pm$ 0.80	4.50 $\pm$ 0.50	4.37 $\pm$ 0.50	4.07 $\pm$ 0.50
CB ₂ (+/+)	3.16 $\pm$ 0.60	3.55 $\pm$ 0.43	3.24 $\pm$ 0.47	3.48 $\pm$ 0.43
CB ₂ (-/-)	4.00 $\pm$ 0.52	4.24 $\pm$ 0.41	3.65 $\pm$ 0.49	3.73 $\pm$ 0.47
Cold allodynia				
Genotype	Vehicle	Gabapentin	PF-3845	JZL184
CB ₁ (+/+)	3.35 $\pm$ 0.30	3.45 $\pm$ 0.20	5.03 $\pm$ 0.52	3.90 $\pm$ 0.28
CB ₁ (-/-)	5.31 $\pm$ 1.59	4.40 $\pm$ 0.83	3.59 $\pm$ 0.45	4.26 $\pm$ 0.59
CB ₂ (+/+)	3.92 $\pm$ 0.50	3.53 $\pm$ 0.37	5.44 $\pm$ 0.63	3.77 $\pm$ 0.39
CB ₂ (-/-)	3.97 $\pm$ 0.45	3.81 $\pm$ 0.27	3.97 $\pm$ 0.50	3.82 $\pm$ 0.42