Blockade of Endocannabinoid-Degrading Enzymes Attenuates Neuropathic Pain


Department of Pharmacology and Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, Virginia (S.G.K., S.T.O., R.A.A., J.L.P., A.H.L.); Skaggs Institute for Chemical Biology and Department of Chemical Physiology, the Scripps Research Institute, La Jolla, California (J.Z.L., B.F.C.); and Skaggs Institute for Chemical Biology and Department of Chemistry, the Scripps Research Institute, La Jolla, California (D.L.B.)

Received April 23, 2009; accepted June 4, 2009

ABSTRACT

Direct-acting cannabinoid receptor agonists are well known to reduce hyperalgesic responses and allodynia after nerve injury, although their psychoactive side effects have dampened enthusiasm for their therapeutic development. Alternatively, inhibiting fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the principal enzymes responsible for the degradation of the respective endogenous cannabinoids, anandamide (AEA) and 2-arachydonylglycerol (2-AG), reduce nociception in a variety of nociceptive assays, with no or minimal behavioral effects. In the present study we tested whether inhibition of these enzymes attenuates mechanical allodynia, and acetone-induced cold allodynia in mice subjected to chronic constriction injury of the sciatic nerve. Acute administration of the irreversible FAAH inhibitor, cyclohexylcarbamic acid 3'-carboxamido-3-phenylheptan-3-yl ester (URB597), or the reversible FAAH inhibitor, 1-oxo-1-[5-(2-pyridyl)-2-yl]-7-phenylheptane (OL-135), decreased allodynia in both tests. This attenuation was completely blocked by pretreatment with either CB1 or CB2 receptor antagonists, but not by the TRPV1 receptor antagonist, capsazepine, or the opioid receptor antagonist, naltrexone. The novel MAGL inhibitor, 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-y1)(hydroxy)methyl)piperidine-1-carboxylate (JZL184) also attenuated mechanical and cold allodynia via a CB1, but not a CB2, receptor mechanism of action. Whereas URB597 did not elicit antiallodynic effects in FAAH(−/−) mice, the effects of JZL184 were FAAH-independent. Finally, URB597 increased brain and spinal cord AEA levels, whereas JZL184 increased 2-AG levels in these tissues, but no differences in either endocannabinoid were found between nerve-injured and control mice. These data indicate that inhibition of FAAH and MAGL reduces neuropathic pain through distinct receptor mechanisms of action and present viable targets for the development of analgesic therapeutics.

Although cannabinoid therapy has been used for thousands of years to treat pain and other ailments, its undesirable psychomimetic effects have dampened enthusiasm for further drug development. Instead, recent research has focused on targeting the effects that reduce neuropathic pain through distinct receptor mechanisms of action and present viable targets for the development of new analgesics (Schlosburg et al., 2009). The endogenous cannabinoid system consists of two cloned cannabinoid receptors (CB1 and CB2), various proposed endocannabinoid ligands, including anandamide (AEA; Devane et al., 1992) and 2-arachidonylglycerol (2-AG; Mechoulam et al., 1995), and the enzymes that regulate the biosynthesis and catabolism of the endocannabinoids. In particular, fatty acid amide hydrolase (FAAH; Cravatt et al., 1996) and monoacylglycerol lipase (MAGL; Blankman et al., 2007) are the primary catalytic enzymes of AEA and 2-AG, respectively.

ABBREVIATIONS: CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; 2-AG, 2-arachidonylglycerol; AEA, anandamide or endogenous cannabinoid system for the development of new analgesics. Instead, recent research has focused on targeting the effects that reduce neuropathic pain through distinct receptor mechanisms of action and present viable targets for the development of new analgesics (Schlosburg et al., 2009). The endogenous cannabinoid system consists of two cloned cannabinoid receptors (CB1 and CB2), various proposed endocannabinoid ligands, including anandamide (AEA; Devane et al., 1992) and 2-arachidonylglycerol (2-AG; Mechoulam et al., 1995), and the enzymes that regulate the biosynthesis and catabolism of the endocannabinoids. In particular, fatty acid amide hydrolase (FAAH; Cravatt et al., 1996) and monoacylglycerol lipase (MAGL; Blankman et al., 2007) are the primary catalytic enzymes of AEA and 2-AG, respectively.
Administration of the irreversible FAAH inhibitor, URB597, or the reversible FAAH inhibitor, OL-135, leads to increased levels of AEA in the brain (Lichtman et al., 2004a; Fogley et al., 2005). In mice, OL-135 produces analgesic effects in the hot-plate, tail-immersion, and formalin tests, via a CB₁ mechanism of action (Lichtman et al., 2004a). Likewise, FAAH(−/−) mice have approximately 10-fold higher levels of anandamide in the brain than wild-type mice (Cravatt et al., 2001). Overall, FAAH(−/−) mice exhibit a CB₁ receptor-mediated analgesic phenotype in a variety of acute and inflammatory pain models (Lichtman et al., 2004b).

Neuropathy often stems from different types of nerve injury, with common symptoms ranging from numbness or mild tingling to moderate to severe pain (Zimmermann, 2001). This pain can present spontaneously, or as increased sensitivity to noxious stimuli (i.e., hyperalgesia), or perceptions of pain in response to non-noxious stimuli (i.e., allodynia). Ligation of the sciatic nerve, known as chronic constriction injury (CCI), is a common model of neuropathic pain in mice that results in thermal hyperalgesia (Lichtman et al., 2004b), mechanical allodynia (Russo et al., 2007), and cold allodynia (Walczak and Beaulieu, 2006). CCI-induced mechanical allodynia and thermal hyperalgesia were attenuated by the mixed CB₁/CB₂ receptor agonist WIN 55,212-2, via both cannabinoid receptor subtypes (La Rana et al., 2008). Likewise, FAAH inhibition reduces neuropathic pain. Repeated injections of URB597 treatment attenuated CCI-induced thermal hyperalgesia and mechanical allodynia, and these effects were completely blocked by either CB₁ or CB₂ receptor antagonists (Russo et al., 2007). In rats, partial nerve ligation also resulted in mechanical allodynia, which was attenuated by OL-135 (Chang et al., 2006). However, in this model, the CB₂ receptor antagonist, SR144528, blocked the antiallodynic effects of OL-135, whereas the CB₁ receptor antagonist, rimonabant, was ineffective. The observation that the µ-opioid antagonist, naloxone, also blocked the antihyperalgesic effects of OL-135 led the authors to conclude that multiple neurochemical mechanisms were involved. The disparate findings between the Chang et al. (2006) and Russo et al. (2007) studies may result from methodological or species differences and warrant further investigation.

Until recently, no selective MAGL inhibitor was available. Although two compounds, URB602 and N-arachidonyl maleimide, inhibited MAGL in the brain, both lack specificity and inhibit other serine hydrolases, including FAAH (Hohmann et al., 2005; Vandevoorde et al., 2007; Burston et al., 2008). The recently developed JZL184 is a highly selective and long-lasting MAGL inhibitor that on systemic administration leads to increased 2-AG levels in the brain and CB₁ receptor-mediated hypoalgesic, hypothermic, and locomotor-suppressant effects in mice (Long et al., 2009), but has yet to be evaluated in a neuropathic pain model.

The present study has four objectives. First, we attempted to reconcile the different effects of URB597 and OL-135 by assessing each compound in the mouse CCI pain model. In addition, we examined whether CB₁, CB₂, TRPV1, and opioid receptor antagonists would block the antiallodynic effects caused by FAAH blockade. Second, because FAAH(−/−) mice subjected to noxious heat stimuli (Lichtman et al., 2004b), we investigated the effects of genetic deletion of FAAH on CCI-induced increases in sensitivity to mechanical and cold stimuli. Third, we sought to determine whether the MAGL inhibitor, JZL184, reduces mechanical and cold allodynia in the CCI model. In addition, we assessed the relative involvement of the CB₁ and CB₂ receptors. Fourth, we sought to quantify the impact of CCI and enzyme inhibition on levels of AEA and 2-AG in the brain and spinal cord.

**Materials and Methods**

**Animals.** Subjects consisted of male C57BL/6J (The Jackson Laboratory, Bar Harbor, ME) that were approximately 8 weeks of age at the beginning of the study. In addition, male FAAH(−/−) and age-matched FAAH(+/+) mice from the Center Transgenic Colony at Virginia Commonwealth University, backcrossed onto a C57BL/6J (13 generations), were used. Mice were housed in a temperature-(20–22°C) and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care-approved facility, with ad libitum access to food and water. Subjects weighed approximately 25 g, and were housed four to six mice per cage and maintained on a 12:12 light cycle. Based on previous studies from our laboratory (e.g., Lichtman et al., 2004; Long et al., 2009), the sample size for all behavioral studies was 7 to 10 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 sacrificed via CO₂ asphyxia, followed by rapid cervical dislocation.

**Chronic Constriction Injury Surgery.** Mice were randomly assigned to sham or CCI treatment groups and were given acetaminophen (2.4 mg/ml in drinking water) from 24 h before surgery to 48 h after surgery. Constant anesthesia was maintained throughout surgery by use of 1.5% isoflurane via inhalation. The right hind leg was shaved and the area swabbed with Betadine solution, then ethanol for antisepsis. An incision was made in the skin posterior to the femur, and the sciatic nerve was isolated after separation of the muscle. The nerve was visualized and ligated twice with use of 4-0 chronic gut suture. The surrounding muscle and skin were then sutured with 6-0 nylon. Mice were allowed to recover on paper towels in a heated cage and observed for approximately 2 h before being returned to the colony.

**Drugs.** URB597 and gabapentin were purchased from Cayman Chemical (Ann Arbor, MI), rimonabant (SR141716A) and SR144528 were obtained from the National Institute on Drug Abuse (Bethesda, MD), naltrexone hydrochloride and capsazepine were purchased from Sigma-Aldrich (St. Louis, MO), and OL-135 was synthesized as described previously (Boger et al., 2005). Drugs were dissolved in a vehicle consisting of ethanol, Alkamuls-620 (sanofi-aventis, Bridge- water, NJ), and saline in a ratio of 1:1:8, and injected intraoperatively in a volume of 10 µl/g body mass. JZL184 was synthesized as described previously (Long et al., 2009) and was dissolved in a vehicle of 4:1 polyethylene glycol (PEG 200) and Tween 80 (Sigma- Aldrich) and injected intraperitoneally at a volume of 4 µl/g body mass (Long et al., 2009). All solutions were warmed to room temperature before injection.

**Nociceptive Testing.** Nociceptive testing began two weeks after surgery. Mice were brought into the testing room, weighted, and allowed to acclimate for at least 1 h before injections. Mice were randomly assigned to a drug treatment regimen by a random number generator. After receiving drug, the mice were placed inside ventilated polycarbonate chambers on an aluminum mesh table and allowed to acclimate to the apparatus for 60 min before testing. Thus, for experiments using URB597 and OL-135, total absorption time was 60 min. In the experiments using JZL184, mice were injected, returned to their home cage, and then placed on the test
Mechanical allodynia was quantified in the contralateral control paw 60 min after drug treatment via von Frey test. B, cold allodynia was tested 90 min after drug treatment via acetone-induced cold allodynia test. C, control paw; D, CCI paw. Data expressed as mean ± S.E.M. (n = 8–10); ** p < 0.01 versus vehicle-treated paw.

Results

CB₁ and CB₂ Receptors Mediate the Antiallodynic Effects of URB597 in von Frey and Acetone-Induced Flinching Assays. CCI caused mechanical allodynia [F(1, 70) = 83; p < 0.0001; Fig. 1A] and cold allodynia [F(1, 70) = 109; p < 0.0001; Fig. 1B]. URB597 dose-dependently attenuated allodynia in mice subjected to CCI. In the CCI paw, there was a main effect of drug treatment on mechanical allodynia [F(4, 39) = 6.5; p < 0.001] and cold allodynia [F(4, 39) = 5.2; p < 0.01], and follow-up comparisons revealed significantly reduced allodynia at the 10 mg/kg dose in both tests (p < 0.01). In both tests, drug treatment had no effect on allodynia in the contralateral, control paw (von Frey, p = 0.92; acetone, p = 0.89). Because the contralateral paws were not significantly affected by treatments in this or any of the experiments described below, these data are not shown in any subsequent figures.

To assess the receptor mechanism of action underlying the antiallodynic effects of URB597 (10 mg/kg), mice were pretreated with rimonabant (3 mg/kg), SR144528 (3 mg/kg), capsazepine (5 mg/kg), or vehicle 10 min before receiving URB597. There was a significant interaction between...
URB597 and pretreatment with rimonabant on mechanical allodynia \[F(1, 32) = 4.329; p < 0.05; \text{Fig. 2A}\] and cold allodynia \[F(1, 32) = 5.944; p < 0.05; \text{Fig. 2B}\], indicating CB\(_1\) receptor involvement. URB597 significantly attenuated allodynia in both tests \((p < 0.05)\), and this effect was completely blocked by pretreatment with rimonabant \((p < 0.05)\). Rimonabant had no effect on allodynia by itself \((\text{von Frey}, p = 0.52; \text{acetone}, p = 0.33)\).

There was a significant interaction between URB597 and pretreatment with the selective CB\(_2\) antagonist SR144528 on mechanical allodynia \[F(1, 28) = 11.560; p < 0.01; \text{Fig. 2C}\] and cold allodynia \[F(1, 28) = 10.146; p < 0.01; \text{Fig. 2D}\]. URB597 significantly attenuated allodynia in both tests \((p < 0.01 \text{ and } 0.001, \text{respectively})\), and this effect was completely blocked by pretreatment with SR144528 \((p < 0.01 \text{ and } 0.001, \text{respectively})\). SR144528 had no effect on allodynia by itself \((\text{von Frey}, p = 0.47; \text{acetone}, p = 0.94)\).

The selective TRPV1 receptor antagonist, capsazepine, had no effect on the mechanical antiallodynic \((\text{URB597 by capsazepine interaction}, p = 0.64; \text{capsazepine main effect}, p = 0.64; \text{Fig. 2E})\) or cold allodynic \((\text{URB597 by capsazepine interaction}, p = 0.58; \text{capsazepine main effect}, p = 0.67; \text{Fig. 2F})\) effects of URB597. URB597 significantly attenuated mechanical \[F(1,28) = 18.9, p < 0.001\] and cold allodynia \[F(1,28) = 50, p < 0.0001\].

CB\(_1\) and CB\(_2\) Receptors Mediate the Antiallodynic Effects of OL-135 in von Frey and Acetone-Induced Flinching Assays. In this experiment, we assessed whether the reversible FAAH inhibitor, OL-135, would yield a similar pattern of effects as the irreversible FAAH inhibitor, URB597, in the CCI model. As shown in Fig. 3, OL-135 \((10 \text{ mg/kg})\) significantly attenuated both mechanical and cold allodynia in mice subjected to CCI. These antiallodynic effects involved both CB\(_1\) and CB\(_2\) receptors, but not TRPV1 or opioid receptors. There was a significant interaction between OL-135 and antagonist pretreatment on mechanical allodynia \[F(4,40) = 9.3; p < 0.0001; \text{Fig. 3A}\] and cold allodynia \[F(4, 40) = 13.4; p < 0.0001; \text{Fig. 3B}\]. OL-135 significantly attenuated allodynia in both tests \((p < 0.05, \text{both tests})\), and this effect was completely blocked by pretreatment with either rimonabant \((3 \text{ mg/kg}; p < 0.01, \text{both tests})\) or SR144528 \((3 \text{ mg/kg}; p < 0.05 \text{ and } 0.01, \text{respectively})\). However, capsazepine did not significantly affect the antiallodynic effects of OL-135 \((\text{von Frey}, p = 0.22; \text{acetone}, p = 0.99)\).

Because Chang et al. (2006) reported that naloxone significantly attenuated the antiallodynic effects of OL-135 in the rat spinal nerve ligation model, we evaluated whether an opioid receptor mechanism was also involved in the antiallodynic effects of this FAAH inhibitor in the mouse CCI model. As shown in Fig. 3, E and F, naltrexone \((1 \text{ mg/kg})\) did not
block the antiallodynic effects of OL-135. There was no significant interaction between OL-135 and naltrexone pretreatment on mechanical allodynia ($p = 0.60$; Fig. 3E) or cold allodynia ($p = 0.66$; Fig. 3F). However, OL-135 significantly attenuated allodynia in both mechanical [$F(1, 26) = 45.6; p < 0.0001$] and cold tests [$F(1, 26) = 21.3; p < 0.0001$], even though this effect was not reversed by pretreatment with naltrexone (von Frey, $p = 0.26$; acetone, $p = 0.94$). In addition, naltrexone given alone had no effect on allodynia (von Frey, $p = 0.10$; acetone, $p = 0.76$).

The Antiallodynic Effects of URB597 and OL-135 Are Mediated through the Suppression of FAAH.

In contrast to the antiallodynic effects of URB597 (10 mg/kg) and OL-135 (10 mg/kg) in the von Frey and acetone-induced flinching assays, we reported previously that FAAH (-/-) mice do not display a reduction in thermal hyperalgesia, as assessed in Hargreaves’s plantar stimulator test (Lichtman et al., 2004b). Accordingly, in the present experiment, we assessed whether this lack of a thermal hyperalgesic phenotype was modality specific or extended to other types of noxious stimuli. FAAH (-/-) mice displayed identical nociceptive behavior as FAAH (+/+), but not FAAH (-/-) in both the von Frey (Fig. 4, A and C) and acetone (Fig. 4, B and D) assays. Thus, genetic deletion and pharmacological inhibition of FAAH lead to different phenotypes in the mouse CCI model of neuropathic pain. To confirm whether the antiallodynic properties of URB597 and
OL-135 were mediated through FAAH, each drug was tested in FAAH(/−/) and FAAH(+/+) mice. There was no effect of genotype on allodynia in vehicle-treated mice (von Frey, \( p = 0.35 \); acetone, \( p = 0.96 \)). In contrast, there were significant interactions between URB597 and genotype for mechanical allodynia [\( F(1, 26) = 10.4; p < 0.05 \); Fig. 4A] and cold allodynia [\( F(1, 26) = 11.7; p < 0.01 \); Fig. 4B]. However, URB597 had no effect in FAAH-deficient mice in either mechanical (\( p = 0.51 \)) or cold (\( p = 0.96 \)) allodynia tests.

Next, OL-135 was compared in FAAH(/−/) and (+/+) mice. In addition, the GABA analog gabapentin (50 mg/kg) was assessed in both genotypes, as a positive control. There was a significant interaction between OL-135/gabapentin and strain on mechanical allodynia [\( F(2, 42) = 6.3; p < 0.01 \); Fig. 4C] and cold allodynia [\( F(2, 42) = 6.7; p < 0.01 \); Fig. 4D]. OL-135 elicited antiallodynic effects in FAAH(+/+) mice, but had no effect in FAAH(/−/) mice in either mechanical (\( p = 0.39 \)) or cold allodynia (\( p = 0.73 \)) tests. Unlike the FAAH inhibitors, gabapentin significantly reduced mechanical and cold allodynia in both genotypes.

**MAGL Inhibitor JZL184 Attenuated Allodynia via CB1 Mechanism of Action.** In the next set of experiments, we evaluated whether the selective MAGL inhibitor, JZL184, would reduce mechanical and cold allodynia in nerve-injured mice. As shown in Fig. 5, JZL184 (16 mg/kg) was efficacious in reducing mechanical and cold allodynia. In addition, these antiallodynic effects were blocked by rimonabant (3 mg/kg), but not by SR144528 (3 mg/kg). Specifically, a significant interaction was found between JZL184 and antagonist pretreatment on mechanical allodynia [\( F(3, 26) = 5.0; p < 0.01 \); Fig. 5A] and cold allodynia [\( F(3, 26) = 15.5; p < 0.0001 \); Fig. 5B]. JZL184 significantly attenuated allodynia in both tests (\( p < 0.05 \)), and these effects were blocked by rimonabant (von Frey, \( p < 0.05 \); acetone, \( p < 0.01 \), but not by SR144528 (von Frey, \( p = 0.65 \); acetone, \( p = 0.46 \)).

Next, to examine whether JZL184 worked via a FAAH-dependent mechanism of action, we evaluated its effects in FAAH(/−/) and (+/+) mice. JZL184 (16 mg/kg) significantly reduced both mechanical [\( F(1, 28) = 42.2; p < 0.0001 \); Fig. 6A] and cold allodynia [\( F(1, 28) = 34.6; p < 0.0001 \); Fig. 6B] in both genotypes. There was no interaction between drug and genotype in either test of mechanical (\( p = 0.82 \)) or cold allodynia (\( p = 0.56 \)).

**Quantification of Endocannabinoid Levels in Brain and Spinal Cord.** The levels of AEA and 2-AG were quantified in whole brain and spinal cord of mice 14 days after CCI or sham surgery. Pretreatment times were 1 h for URB597 (10 mg/kg) and 2 h for JZL184 (16 mg/kg), along with their respective vehicles. Because there were no significant differences between the ethoxylated castor oil (Alkamuls-620)/ethanol/saline and polyethylene glycol/Tween 80 vehicles, for AEA or 2-AG in the brain (unpaired \( t \) tests, respective \( p \) values = 0.17 and 0.53) or spinal cord (unpaired \( t \) tests, respective \( p \) values = 0.52 and 0.46), these control groups were collapsed. The FAAH inhibitor, URB597, significantly increased AEA levels in brain [\( F(2, 29) = 18.8; p < 0.0001 \); Table 1] and spinal cord [\( F(2, 25) = 67.2; p < 0.0001 \)] of both CCI and sham-operated mice. URB597 had no effect on 2-AG in either tissue. The MAGL inhibitor, JZL184, significantly increased 2-AG levels in brain [\( F(2, 29) = 20.7; p < 0.0001 \)] and spinal cord [\( F(2, 25) = 14.9; p < 0.0001 \)] of both CCI and sham-operated mice, but had no effect on AEA in either matrix. Overall, CCI did not affect AEA or 2-AG levels in whole brain (respective \( p \) values = 0.59 and 0.40) or whole spinal cord (respective \( p \) values = 0.55 and 0.35).

**Discussion**

Inhibition of FAAH by either irreversible (URB597) or reversible (OL-135) FAAH inhibitors attenuated mechanical and cold allodynia in the CCI model. These antiallodynic effects were blocked by pretreatment with either the CB1 receptor antagonist/inverse agonist, rimonabant, or the CB2 receptor antagonist, SR144528. Genetic FAAH-deficient mice showed no difference in CCI-induced allodynia, compared with wild-type mice. URB597 and OL-135 were ineffective in FAAH(/−/) mice, indicating that the observed antiallodynic effects were due to FAAH modulation. The novel MAGL inhibitor, JZL184, also attenuated both mechanical and cold allodynia, although these effects were blocked by rimonabant, but not SR144528 pretreatment. Finally, URB597 increased AEA, but not 2-AG, levels in whole brain and spinal cord, whereas JZL184 elicited the opposite pattern of effects in both matrices.

URB597 has been shown previously to attenuate CCI-induced mechanical allodynia and thermal hyperalgesia.
was virtually identical in both genotypes (Lichtman et al., 2004b). Thus, the stimulus modality (i.e., radiant heat versus touch or cold) does seem to be responsible for the lack of a phenotype difference. FAAH(+/−) mice may undergo adaptive changes during development that alter the neuroimmune response to neuropathy. Alternatively, adaptive changes in FAAH knockout mice may differentially affect the nociceptive pathways activated by nerve injury. For example, nerve injury may affect neural cannabinoid receptor function differently in FAAH knockout mice, compared with wild-type mice. In support of this hypothesis, FAAH(−/−) mice no longer showed phenotypic hypoalgesia in the tail immersion or hot-plate tests after CCI surgery (Lichtman et al., 2004b). Genetic FAAH knockout mice were unresponsive to both URB597 and OL-135, but gabapentin or the MAGL inhibitor, JZL184, elicited full antiallodynic effects in these animals. These data confirm that the antiallodynic effects of URB597 and OL-135 are mediated through a FAAH-dependent mechanism. Conversely, these findings indicate that the antiallodynic effects of FAAH inhibition were reversed by CB1 and CB2 receptor antagonists. Although CB2 receptor agonists have analgesic effects, the functional role of the CB2 receptor in pain circuits is not well understood, even though these receptors are up-regulated in immune cells after injury. For example, microglia show increased CB2 re-
ceptron expression in mice subjected to CCI compared with noninjured mice (Zhang et al., 2003). Both CB1 and CB2 receptors have been implicated in modulating inflammatory pain models (Clayton et al., 2002). After injury, neuropathy is modulated by glia in the dorsal spinal cord, including activated microglia and astrocytes (Ledeboer et al., 2005; Watkins et al., 2007). These cells produce chemokines, which recruit other immune cells to infiltrate the injured tissue, and proinflammatory cytokines including interleukin-1, interleukin-6, and tumor necrosis factor-α. Further nerve injury results, coupled with noxious neural stimulation, resulting in pain perception by the host (Samad et al., 2001; McMahon et al., 2005; Watkins et al., 2007). Blocking this proinflammatory cytokine cascade results in decreased pain after CCI (Milligan et al., 2006). Given the neuroinflammatory nature of the nerve injury in the CCI model, it is not surprising that both CB1 and CB2 receptors also play a role in modulating neuropathic pain (Russo et al., 2007; Jhaveri et al., 2008; La Rana et al., 2008). The findings of Russo et al. (2007), taken together with the present data, indicate that both CB1 and CB2 receptors are involved in the antiallodynic effects of FAAH inhibition. One explanation for the observation that each cannabinoid receptor plays a necessary role in the antiallodynic effects of FAAH inhibitors is that CB1 and CB2 receptors act at different levels of serial nociceptive and/or inflammatory pathways. The CB2 receptor is expressed predominantly on cells of the immune system, at approximately 10 to 100 times that of CB1 (Galiegue et al., 1995). However, with the development of improved antibodies, this receptor has now been identified in brain stem neurons (Van Sickle et al., 2005). Although not typically expressed at high levels in healthy tissues, CB2 receptors are up-regulated in diseased nervous tissue (Wotip caused at high levels in healthy tissues, CB2 receptors are up-regulated in diseased nervous tissue (Woth-666). Thus, CB2 receptor stimulation may decrease al- lodynia at the level of peripheral nociceptors, spinal nerves, and afferents, or supraspinally. However, CB2 receptors are most probably involved in neural tissue. Peripheral deletion of CB2 on nociceptors (with CB1 preserved in the central nervous system) blocked the analgesic effects of locally and systemically administered cannabinoids (Agarwal et al., 2007).

We were surprised to find that 2-AG seems to attenuate allodynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-
dynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-
dynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-
dynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-
dynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-
dynia via a CB1 receptor mechanism, with no apparent contribution from the CB2 receptor. Whether these antiallo-

References

Endocannabinoids Attenuate Neuropathic Pain 909

References


Ledeboer A, Gamanos M, Lai W, Martin D, Maier SF, Watkins LR, and Quan N

910 Kinsey et al.

910 Kinsey et al.

2:293–308.

126:102–114.


ropathic pain after oral administration in mice. J Pharmacol Exp Ther


21:131–146.


17:2750–2764.

429:23–37.

Address correspondence to: Dr. Aron Lichtman, PO Box 980613, Rich-
mond, VA 23298. E-mail: alichtma@vcu.edu.