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Fatty acid amide hydrolase blockade attenuates the development of collagen-induced arthritis and related thermal hyperalgesia in mice

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ABSTRACT

Fatty acid amide hydrolase (FAAH) is the primary degradative enzyme of the endocannabinoid anandamide (*N*-arachidonoylethanolamine), which activates cannabinoid CB_1 and CB_2 receptors. FAAH disruption reduces nociception in a variety of acute rodent models of inflammatory pain. The present study investigated whether these actions extend to the chronic, collagen-induced arthritis (CIA) model. We investigated the anti-arthritic and anti-hyperalgesic effects of genetic deletion or pharmacological inhibition of FAAH in the CIA model. FAAH (−/−) mice, and FAAH-NS mice that express FAAH exclusively in nervous tissue, displayed decreased severity of CIA and associated hyperalgesia. These phenotypic anti-arthritic effects were prevented by repeated daily injections of the CB_2 receptor antagonist, SR144528, but not the CB_1 receptor antagonist rimonabant. Similarly, repeated administration of the FAAH inhibitor URB597 reduced CIA severity, and acute administration of rimonabant, but not SR144528, blocked the anti-hyperalgesic effects of prolonged FAAH inhibition, suggesting that prolonged CB_2 receptor activation reduces the severity of CIA, whereas acute CB_1 receptor activation reduces CIA-induced hyperalgesia. In contrast, acute administration of URB597 elicited a CB_1 receptor-dependent anti-hyperalgesic effect. The observed anti-arthritic and anti-hyperalgesic properties of FAAH inhibition, coupled with a lack of apparent behavioral alterations, suggest that endocannabinoid modulating enzymes offer a promising therapeutic target for the development of novel pharmacological approaches to treat rheumatoid arthritis and associated hyperalgesia.

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1. Introduction

Increased pain sensitivity is a common and debilitating symptom of inflammatory disorders (Dray and Bevan, 1993). The endogenous cannabinoid (endocannabinoid) system, consisting of two known cannabinoid receptors (i.e., CB_1 and CB_2 (Gerard et al., 1991; Matsuda et al., 1990)), endogenously produced ligands including *N*-arachidonoylethanolamine (Devane et al., 1992), and 2-arachidonoylglycerol (2-AG; (Mechoulam et al., 1995; Sugiura et al., 1995)), and the enzymes regulating ligand biosynthesis and degradation (Elphick and Egertova, 2005), is believed to play a significant role in modulating physiological responses to inflammation and pain. Accordingly, the endocannabinoid system holds promise as a therapeutic target to treat peripheral and central inflammatory disorders (Booker et al., 2011; Guindon and Hohmann, 2008; Schlosburg et al., 2009b). In support of this assertion, endocannabinoids have been found in synovial fluid of patients with end stage osteoarthritis and rheumatoid arthritis, and CB_1 and CB_2 receptors are present in synovial tissue (Richardson et al., 2008).

Cannabis extracts and cannabinoid receptor agonists have long been known to elicit analgesic and anti-inflammatory actions in humans and laboratory animals (Kosersky et al., 1974; Reynolds, 1890; Sofia et al., 1973); however, their therapeutic utility has been greatly limited by the occurrence of psychotropic side effects. Several studies have demonstrated robust anti-inflammatory and anti-hyperalgesic phenotypes following genetic or pharmacological disruption of fatty acid amide hydrolase (FAAH) (Ahn et al., 2009; Cravatt et al., 2001; Holt et al., 2005; Karsak et al., 2007; Kinsey et al., 2011a; Lichtman et al., 2004a; Lichtman et al., 2004b; Massa et al.,

Abbreviations: AEA, anandamide, N-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CIA, Collagen-induced arthritis; FAAH, fatty acid amide hydrolase; NSAID, Nonsteroidal anti-inflammatory drug; OEA, *N*-oleoylethanolamide; PEA, *N*-palmitoylethanolamide; Rim, Rimonabant,N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl; SR2, SR144528, N-[(1S)-endo-1,3,3,-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide; THC, Δ^9-tetrahydrocannabinol; TRPV1, transient receptor potential cation channel, subfamily V, member 1; URB597, cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-yl ester.

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2004; Naidu et al., 2010; Russo et al., 2007), the principal degradative enzyme for anandamide and other bioactive fatty acid amides (FAAs) (Cravatt et al., 1996; Maurelli et al., 1995; Ueda et al., 1995). The molecular basis for the anti-inflammatory and anti-hyperalgesic effects of FAAH disruption continues to be an area of interest (Booker et al., 2011; Kinsey et al., 2010; Rahn and Hohmann, 2009).

FAAH-disrupted rodents have been investigated in a variety of inflammatory pain models including carrageenan, lipopolysaccharide, and complete Freund's adjuvant models (Ahn et al., 2009; Cravatt et al., 2001; Cravatt et al., 2004; Holt et al., 2005; Jayamanne et al., 2006; Lichtman et al., 2004b; Naidu et al., 2010). In the present study, we investigated the role of the endogenous cannabinoid system in the murine collagen-induced arthritis (CIA) assay, which results in a progressive inflammation of the joints, as well as degeneration of both cartilage and bone (Campbell et al., 1998; Moore, 2003). Several pathologic features found in CIA resemble symptoms that are not only found in rheumatoid arthritis patients, but are also responsive to similar pharmacological treatments (Courtenay et al., 1980; Trentham, 1982; Williams, 1998). The development of CIA occurs over a prolonged period of time, and closely models chronic inflammatory disorders in humans (Bluml et al., 2011). Of significance, repeated administration of cannabidiol, a major cannabinoid constituent of *Cannabis sativa* that lacks psychoactive properties or affinity for CB_1 or CB_2 receptors, reduces the severity of CIA (Malfait et al., 2000). However, no published studies have evaluated the role of endogenous cannabinoids in this chronic model of arthritis.

There were three objectives in the present study. First, we used complementary genetic and pharmacological approaches to investigate whether FAAH blockade reduces collagen-induced arthritis and CIA associated hyperalgesia. Second, we assessed the relative contribution of FAAH in the nervous system using neural specific transgenic FAAH mice, in which this enzyme is expressed exclusively on neural tissue. Third, we used CB_1 and CB_2 receptor antagonists to determine whether the anti-arthritic and anti-hyperalgesic phenotypes of FAAH-deficient mice require cannabinoid receptors.

2. Material and methods

2.1. Subjects

Male DBA1/J mice (Jackson Laboratory, Bar Harbor, ME) were used to investigate the pharmacological effects of the FAAH inhibitor, URB597. In addition, two types of genetically altered mice were used (Center Transgenic Colony at Virginia Commonwealth University). First, male and female FAAH (−/−) mice were used to examine the impact of FAAH deletion on CIA, as compared with their littermate FAAH (+/+) mice. Second, a transgenic mouse model was used in which the central and peripheral FAAH systems had been functionally uncoupled. Mice expressing FAAH specifically in the nervous system (FAAH-NS mice) were generated by crossing FAAH (−/−) mice with transgenic mice that express FAAH under the neural specific enolase promoter (Cravatt et al., 2004). FAAH-NS possess wild type levels of anandamide and other FAAs in the brain and spinal cord, but significantly elevated concentrations of these lipid transmitters in peripheral tissues. FAAH-NS mice were compared to two kinds of littermate control groups, global FAAH (−/−) mice and FAAH (+/−) mice; the latter possesses wild type levels of FAAs (Cravatt et al., 2004). Each of these three genotypes was derived from breeding pairs that included a FAAH (−/−) mouse and a FAAH (+/−) mouse that expressed the FAAH transgene. All experiments involving genetically altered mice used male and female mice that were derived from breeding pairs that were backcrossed onto a DBA1 background for five or six generations. The genotype of each genetically altered mouse was confirmed by RT-PCR. Mice were counterbalanced across treatment groups to control for possible sex differences in the development and severity of CIA, and concomitant CIA-induced hyperalgesia.

All mice weighed 20–30 g and were housed four per cage in a temperature-controlled (20–22 °C), AAALAC accredited facility. Food and water were available ad libitum. All animal protocols were approved by the VCU Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs

URB597 (FAAH inhibitor; Cayman Chemicals, Ann Arbor, MI), rimonabant (SR1; CB_1 receptor antagonist; NIDA, Rockville, MD), and SR144528 (SR2; CB_2 receptor antagonist; NIDA, Rockville, MD) were dissolved in a vehicle that consisted of 1:1:18 ethanol:alkamuls-620 (Rhone-Poulenc, Princeton, NJ):normal saline. All drugs were administered intraperitoneally (i.p.) in a volume of 10 μl/g body weight.

2.3. Collagen-induced arthritis (CIA) model

For CIA induction, mice were immunized with an injection of 100 μg of chicken type II collagen (Sigma) in 50 μl of 0.05 M acetic acid, emulsified in an equal volume of complete Freund's adjuvant, distal to the fur on the sole of the left hind paw. Mice were challenged with a booster injection of an equal volume of the same collagen preparation in incomplete Freund's adjuvant (i.p.) 21 days later. All four paws were examined twice weekly for arthritic signs as follows: 0, normal; 1, erythema and mild swelling confined to the ankle joint or toes; 2, erythema and mild swelling extending from the ankle to the midfoot or ankle joint; 3, erythema and moderate swelling extending from the ankle to the metacarpal/metatarsal joints; 4, erythema and severe swelling encompassing the ankle, foot, and digits. The scores for each of the four limbs were summed for each mouse, resulting in a composite arthritis score with a maximum of 16 total points. All arthritic mice were randomly distributed in various treatment groups and were monitored and treated daily throughout the 15-day period of heterologous CIA. Experimenter was blinded to treatment conditions.

In experiments examining the impact of repeated drug administration on CIA development, URB597 (10 mg/kg) or vehicle was administered once or twice daily from days 3 to 15 after the booster collagen injection. In order to determine whether the FAAH (−/−) anti-arthritic phenotype was mediated through a cannabinoid receptor mechanism of action, mice were treated repeatedly with rimonabant (3 mg/kg), SR144528 (3 mg/kg), or vehicle. Injections were given at approximately 0900 and 1700 h each day.

At the conclusion of the experiments, mice were humanely euthanized as specified by IACUC guidelines. The hind paws of a subset of FAAH (−/−) and (+/+) mice were dissected, fixed in 10% neutral buffered formalin, and sent to Premier Laboratories (Boulder, CO) for histology. The knees were sectioned from the limb and decalcified in 10% formic acid. The tissue was then embedded in paraffin wax blocks, sectioned to 8-μm thickness with a microtome, and stained with toluidine blue. Arthritic changes in the knee and ankle joints were assessed using a scoring system of 0–5, 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe for inflammation, pannus formation, cartilage damage, and bone resorption, as detailed in Table 1. The scores for both ankles and knees were summed for each mouse, resulting in a composite arthritis score with a maximum of 20 total points for each measure. In order to increase objectivity and consistency, dependent measures were assessed by a trained technician who was blinded to the treatments.

2.4. Nociceptive tests

Nociceptive behavior was evaluated 15 days after the booster injection of collagen with the hot plate and tail immersion tests. The

thermal stimulus in each assay was maintained at 52.0 °C, which is below the threshold to elicit phenotypic analgesic responses in FAAH (−/−) mice under baseline conditions (Cravatt et al., 2001). In the hot plate test, the latency to jump or lick/shake a hind paw was scored. A 30 s cutoff time was used to avoid the possibility of tissue damage. In the tail immersion test, each mouse was placed head first into a small bag fabricated from absorbent under pads (VWR Scientific Products; 4 cm diameter, 11 cm length), leaving the tail exposed. The experimenter gently held the mouse, quickly dipped the tail approximately 1 cm into the water bath, rapidly removed it, and wiped it dry with a Kimwipe, and then immersed the tail approximately 1 cm into the water bath and scored the latency for the animal to withdraw its tail from the water to the nearest 0.1 s. Because water transfers heat more quickly than the hot plate, a shorter cutoff time (10 s) was used to reduce the possibility of tissue damage.

In experiments assessing the anti-hyperalgesic effects of URB597 (10 mg/kg), nociceptive behavior was assessed before and 60 min after injection. In separate groups of animals, cannabinoid receptor mechanisms of action were evaluated by administering rimonabant (3 mg/kg) or SR144528 (3 mg/kg) 10 min before URB597 (10 mg/kg), or vehicle. Drug doses were based on pilot experiments and previous literature on anti-hyperalgesic effects of URB597 (Kinsey et al., 2009; Naidu et al., 2009; Naidu et al., 2010). The experimenter was blinded to treatment conditions.

2.5. Statistical analysis

Data were analyzed using one-way or two-way ANOVA, with the Newman–Keuls or Dunnett's test used for post hoc analyses. Planned comparisons between genotypes were conducted using Student's *t*-test. All differences were considered significant if $p<0.05$.

3. Results

3.1. FAAH (−/−) mice have an anti-arthritic phenotype

Mice lacking FAAH, which have elevated levels of anandamide as well as non-cannabinoid FAAs (Cravatt et al., 2001), displayed a striking decrease in the severity of CIA on day 15 following collagen re-exposure. Histological examination of knee and ankle joints revealed that control mice showed severe joint damage that included statistically significant increases in bone and articular cartilage erosion, pannus formation, and infiltration of inflammatory cells in the knee and ankle joints (Fig. 1a). Scoring criteria for each paw are summarized in Table 1. For each measure, FAAH (−/−) mice developed a statistically significant less severe pathological response to collagen than FAAH (+/+) mice. Macroscopic inspection of limbs revealed a significant interaction between genotype and day on arthritic index score [$F(5,80)=2.9$, $p<0.05$] in which FAAH (−/−) mice showed a significant decrease in arthritis severity compared to FAAH (+/+) mice from days 9 to 15 (Fig. 1b). As shown in Fig. 1c, collagen led to hyperalgesic responses in FAAH (+/+), while FAAH (−/−) mice exhibited anti-hyperalgesic phenotypes in the hot plate [$F(1,16)=13.6$, $p<0.01$] and tail immersion tests [$F(1,16)=15.9$, $p<0.01$; Fig. 1c] on day 15.

3.2. Deletion of FAAH in non-neuronal tissue reduces arthritis severity

FAAH regulates anandamide and other FAAs in the nervous system and non-neuronal tissues (Cravatt et al., 2004; Elphick and Egertova, 2005), either of which could regulate arthritis development. To distinguish between the roles of neuronal and non-neuronal FAAH, we evaluated the development of CIA in transgenic mice that express FAAH exclusively in the nervous system (i.e., FAAH-NS mice). FAAH-NS mice express low, wild type levels of anandamide and other fatty acid amides in the nervous system, but have significantly elevated levels of these lipid signaling molecules in non-neuronal tissues such as liver and testes (Cravatt et al., 2004), and presumably also the ankle and knee joints. In the present study, a significant interaction was found between genotype and day for arthritic scores [Fig. 2a; $F(10,70)=2.8$, $p<0.001$]. FAAH (−/−) mice showed a significant attenuation in the development of arthritis (Fig. 2a and b) as well as significant reductions in CIA-induced hyperalgesia in both the hot plate [Fig. 2c; $F(2,14)=12.2$, $p<0.001$] and tail withdrawal assays [Fig. 2d; $F(2,14)=15.0$, $p<0.001$], compared to their littermate FAAH (+/−) control mice. Strikingly, FAAH-NS mice retained the anti-arthritic (Fig. 2a and b) and anti-hyperalgesic phenotypes (Fig. 2c and d), implicating the involvement of non-neuronal FAAs. Because FAAH (+/−), FAAH (−/−), and FAAH-NS mice that did not receive collagen treatment displayed nearly identical nociceptive latencies in both tests, their data were collapsed in Fig. 2c and d.

Table 1
Histopathologic scoring was performed by an independent examiner blinded to treatment condition. Individual mouse knees and ankles were given scores of 0–5 for inflammation, pannus formation, cartilage damage and bone resorption according to these criteria.

Score	Inflammation
0	Normal
1	Minimal infiltration of inflammatory cells in periarticular tissue
2	Mild infiltration
3	Moderate infiltration with moderate edema
4	Marked infiltration with marked edema
5	Severe infiltration with severe edema
Score	Pannus formation
0	Normal
1	Minimal infiltration of pannus in cartilage and subchondral bone
2	2 = Mild infiltration
3	3 = Moderate infiltration
4	4 = Marked infiltration
5	5 = Severe infiltration
Score	Cartilage damage
0	Normal
1	Minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption
2	Mild loss of toluidine blue staining with focal mild (superficial) chondrocyte loss and/or collagen disruption
3	Moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption
4	Marked loss of toluidine blue staining with multifocal marked (depth to deep zone) chondrocyte loss and/or collagen disruption
5	Severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption
Score	Bone resorption
0	Normal
1	Minimal: small areas of resorption, not readily apparent on low magnification, rare osteoclasts
2	Mild: more numerous areas of, not readily apparent on low magnification, osteoclasts more numerous
3	Moderate: obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous
4	Marked: full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts
5	Severe: full thickness defects in cortical bone, often with distortion of profile of remaining cortical surface, marked loss of medullary bone, numerous osteoclasts

3.3. The FAAH (−/−) anti-arthritic phenotype requires CB_2 receptors

We next tested whether cannabinoid receptors mediate the anti-arthritic and anti-hyperalgesic FAAH (−/−) phenotypes. Accordingly, FAAH (−/−) mice and their littermate FAAH (+/+) control mice were given two daily i.p. injections of vehicle, the CB_1 receptor antagonist, rimonabant (3 mg/kg), or the CB_2 receptor antagonist, SR144528

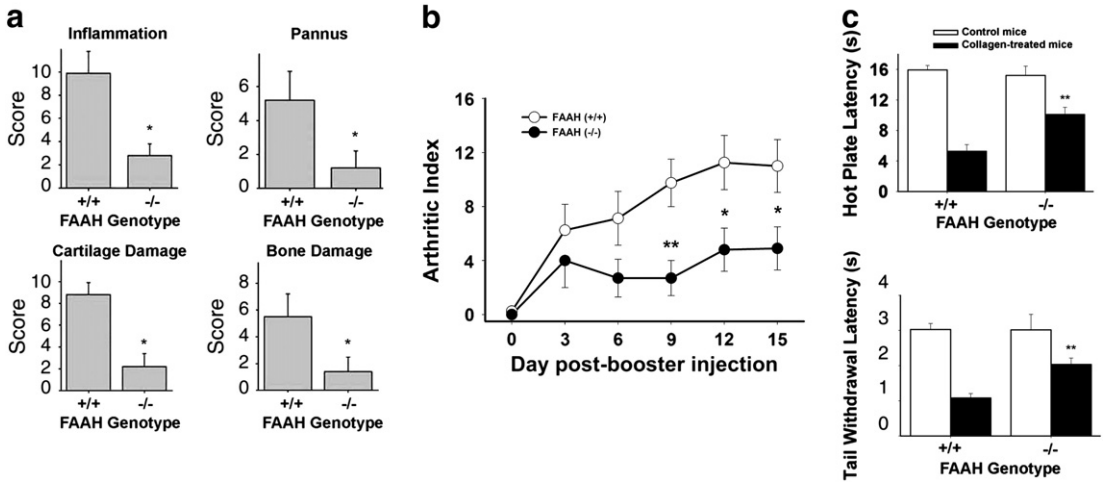

Fig. 1. FAAH deletion reduces the severity of collagen-induced arthritis. (a,b) FAAH (−/−) mice showed significant reductions in arthritis development and severity, as compared to wild type mice. (c) FAAH (−/−) mice displayed phenotypic decreases in the magnitude of hot plate and tail immersion thermal hyperalgesic responses associated with collagen-induced arthritis. *p<0.05; **p<0.05 vs. corresponding littermate FAAH (+/+) control mice treated with incomplete Freund's adjuvant. Data depicted as means ± SEM (n = 8–10 male and female mice per group).

(3 mg/kg), from days 3 to 15 after the booster injection of collagen. A significant interaction was found between cannabinoid receptor antagonist treatment and day in FAAH (−/−) mice [$F(10,70) = 2.7$, $p<0.01$]. Repeated administration of SR144528 prevented the anti-arthritic phenotype in FAAH (−/−) mice, but rimonabant-treated FAAH (−/−) mice did not differ from the vehicle-treated FAAH (−/−) mice (Fig. 3a). In FAAH (+/+) mice, neither cannabinoid receptor antagonist altered the development of CIA (data not shown). Representative paws of collagen-treated and control mice in each of the conditions are shown in Fig. 3b.

3.4. Pharmacological inhibition of FAAH reduces CIA

In the final set of experiments, we evaluated whether pharmacologic inhibition of FAAH reduces the development of CIA and attenuates CIA-induced hyperalgesia. Repeated administration of URB597 (10 mg/kg), beginning on the third day and continuing through the 15th day after the collagen booster injection, significantly reduced CIA progression [Fig. 4a; $F(10,95) = 3.3$, $p<0.001$ for interaction between URB597 treatment and day]. Representative paws in each of the conditions are depicted in Fig. 4b. Acute injection

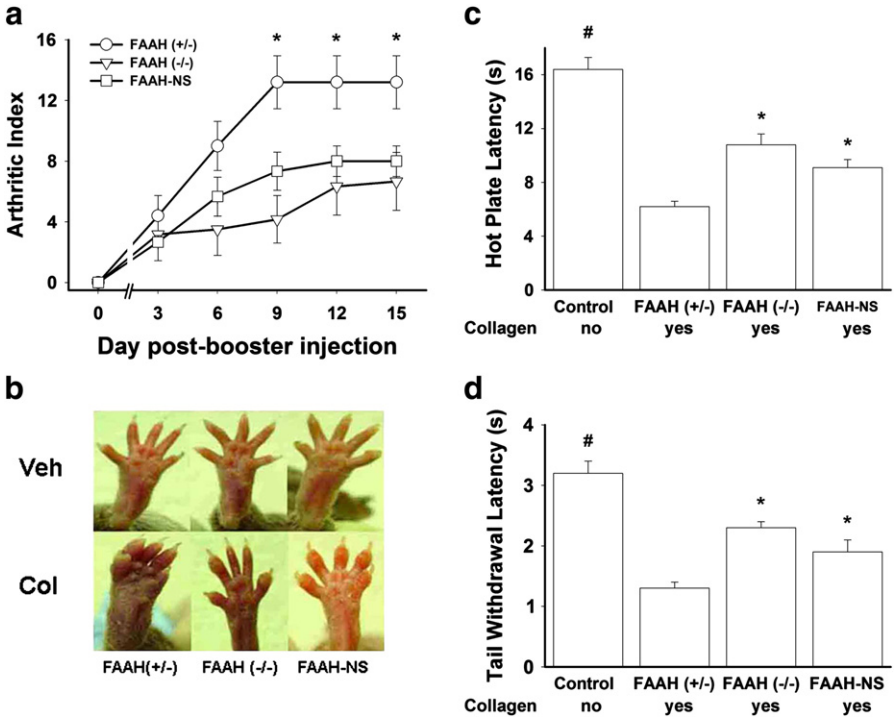

Fig. 2. Deletion of non-neuronal FAAH produces an anti-arthritic phenotype. (a) FAAH-NS mice, which express FAAH exclusively in the nervous system, showed an equivalent reduction in collagen-induced arthritis as global FAAH (−/−) mice compared to FAAH (+/−) mice (controls). *p<0.05 compared to FAAH (−/−) and FAAH-NS mice. (b) Photographs of representative arthritic and non-arthritic FAAH (+/−), (−/−), and -NS mouse hind paws. Both FAAH-NS and (−/−) mice displayed an anti-arthritic phenotype compared to the FAAH (+/−) control mice. Vehicle was incomplete Freund's adjuvant. (c, d) FAAH-NS and FAAH arthritic (−/−) mice displayed antihyperalgesic phenotypes in the hot plate and tail immersion assays compared to FAAH (+/−) mice. FAAH-NS, FAAH (−/−), and FAAH (+/−) mice were littermates. *p<0.05 compared to FAAH (+/−) mice; #p<0.05 compared to mice treated with collagen. Data depicted as means ± SEM (n = 6 male and female mice per group).

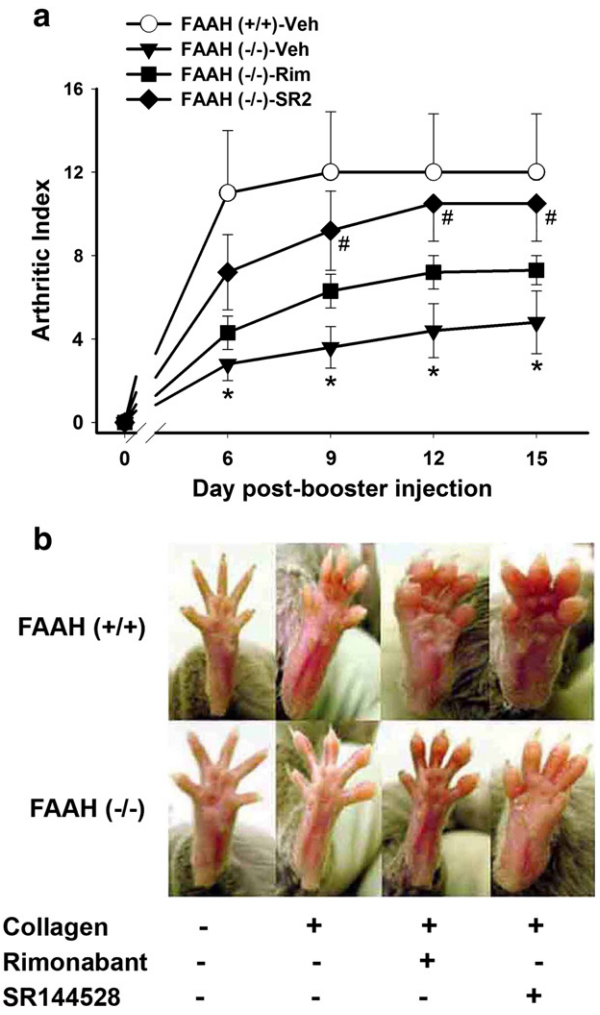

Fig. 3. CB_2 receptors mediate the FAAH (−/−) anti-arthritic phenotype. (a) Chronic treatment (days 3 to 15 following the booster collagen injection) of the CB_2 receptor antagonist SR144528 (SR2; 3 mg/kg, i.p., twice daily), but not the CB_1 receptor antagonist rimonabant (Rim; 3 mg/kg, i.p., twice daily), prevented the FAAH anti-arthritic phenotype in collagen-treated mice. (b) Photographs of representative mouse hind paws showing FAAH (+/+) and (−/−) non-arthritic (Veh+Veh) and arthritic mice that were given two daily injections of vehicle, rimonabant (Rim, 3 mg/kg), or SR144528 (SR2, 3 mg/kg) for 12 days. Chronic SR144528 attenuated the anti-arthritic phenotype in FAAH (−/−) mice, whereas chronic rimonabant treatment did not significantly alter this response. *$p<0.05$ as compared to corresponding littermate FAAH (+/+) control mice (planned comparisons). #$p<0.05$ as compared to vehicle-treated FAAH (−/−) mice (Dunnett's test). Data depicted as means ± SEM (n = 5–6 male and female FAAH (−/−) mice per group and 4 vehicle-treated FAAH (+/+) male and female mice).

of URB597 significantly attenuated CIA-induced hyperalgesia in the hot plate test [Fig. 4c; $F(4,28) = 11.3$, $p<0.001$ for interaction between antagonist and URB597] and the tail immersion test [Fig. 4d; $F(4,28) = 11.8$, $p<0.001$ for interaction between antagonist and URB597]. Rimonabant, but not SR144528, blocked the anti-hyperalgesic effects of URB597, implicating the involvement of CB_1 receptors. In the absence of URB597, neither antagonist altered nociceptive latencies in arthritic control mice (data not shown).

4. Discussion

Rheumatoid arthritis is a debilitating autoimmune disease that affects approximately 1% of the human population and is characterized by inflammation of multiple joints, synovial thickening, ankylosis, joint swelling, and joint pain (Lee and Weinblatt, 2001). The chronic nature of this disease results in destruction of joint cartilage, erosion, loss of function, and reduced quality of life. First-line pharmacological treatments for rheumatoid arthritis include steroids and nonsteroidal anti-inflammatory drugs (NSAIDs), both of which are associated with serious side effects. Similarly, disease modifying anti-rheumatic drugs, such as methotrexate, gold salts, and anti-TNF antibodies also elicit considerable toxic effects (Bongartz et al., 2006; Lee and Weinblatt, 2001). Thus, there is a substantial need for new pharmacological approaches that curtail the progression of the disease and also reduce the pain, without producing serious side effects associated with existing treatments.

One promising target to treat inflammatory diseases is FAAH, the enzyme responsible for the degradation of the endocannabinoid anandamide, as well as an array of other lipid signaling molecules. Indeed, FAAH (−/−) mice and rodents treated with FAAH inhibitors show anti-inflammatory and analgesic effects in a variety of models of inflammation and inflammatory pain (Ahn et al., 2009; Cravatt et al., 2001; Ezzili et al., 2011; Holt et al., 2005; Jayamanne et al., 2006; Karsak et al., 2007; Lichtman et al., 2004a; Lichtman et al., 2004b; Naidu et al., 2010; Wise et al., 2008). The present study is the first of its kind demonstrating that genetic deletion or pharmacological inhibition of FAAH possesses efficacy in a chronic murine arthritis model.

FAAH inhibition or deletion attenuated both inflammation and inflammation-induced thermal hyperalgesia. Prolonged pharmacological inhibition of the CB_2 receptor, but not the CB_1 receptor, blocked the anti-arthritic FAAH (−/−) phenotype. In contrast, the anti-hyperalgesic phenotypes of FAAH-disrupted mice required acute activation of CB_1, but not CB_2, receptors. These effects are consistent with previous observations showing that CB_1 receptors are located at multiple sites throughout the nervous system (Herkenham et al., 1991), including peripheral terminals of nociceptors (Agarwal et al., 2007), the dorsal horn of the spinal cord, and key brain areas associated with pain (Lichtman et al., 1996; Martin et al., 1995; Meng et al., 1998; Yaksh, 1981), which play a role in cannabinoid-induced antinociception.

The observation that FAAH-NS mice, which express FAAH exclusively in nervous tissue, that is in cells containing a neural-specific enolase (Cravatt et al., 2004), exhibited reductions in the development of arthritis equivalent in magnitude to those observed in global FAAH (−/−) mice suggests that non-neuronal FAAs mediate the anti-arthritic phenotype. Accordingly, the presence or absence of FAAH expressed in peripheral nerves that innervate the knee or ankle joints, as well as the metacarpals/metatarsals, would influence the peripheral breakdown of anandamide in these regions. These findings are consistent with a recent report showing that the peripheral FAAH inhibitor URB937 possesses anti-hyperalgesic, anti-allodynic, and anti-inflammatory properties (Clapper et al., 2010).

Reports examining the tonic involvement of endocannabinoids in pain have been mixed, with some research showing that rimonabant produces hyperalgesia in wild type animals (Calignano et al., 1998; Richardson et al., 1997), and work finding no changes from basal nociceptive behavior (Beaulieu et al., 2000). On the other hand, exposure to prolonged foot shock elicits an endocannabinoid-mediated, stress-induced analgesia that is further augmented by inactivation of FAAH or monoacylglycerol lipase, the primary enzyme responsible for 2-AG metabolism (Hohmann et al., 2005). Accordingly, it is plausible that the endogenous cannabinoid system is typically quiescent, but becomes active in response to stressors. In the present study, there was no apparent influence of endocannabinoid signaling over nociception or inflammation in the absence of FAAH disruption. Also, FAAH (+/+) mice as well as FAAH (+/−) mice did not differ from DBA1/J mice in terms of CIA development or CIA-induced hyperalgesia. Thus, FAAH may normally curtail endocannabinoid dampening of inflammatory responses, making it an attractive target to treat pain and inflammation.

The observations that prolonged antagonism of CB_2 receptors prevents the FAAH (−/−) anti-arthritic phenotype, and that acute

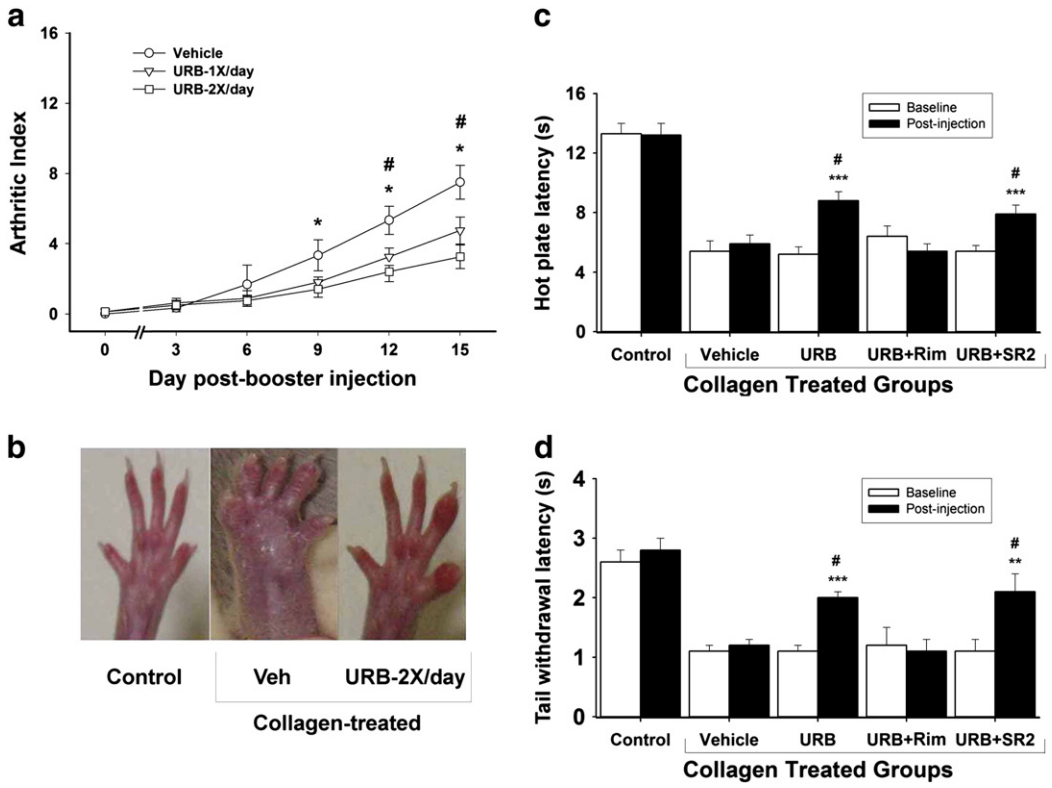

Fig. 4. Chronic and acute effects of the FAAH inhibitor URB597 (URB) on collagen-induced arthritis. (a) URB (10 mg/kg, i.p.) administered twice daily from days 3 to 15 post-booster injection, significantly diminished arthritis severity, while URB administered once daily only partially blocked these effects. *$p<0.05$ vs. URB-2X/day mice; #$p<0.05$ vs. URB-1X/day mice (Newman–Keuls). (b) Photographs of representative mouse hind paws showing chronic URB administration (two injections per day) reduced arthritis severity compared to arthritic mice given daily injections of vehicle. Acute administration of the FAAH inhibitor URB597 (URB; 10 mg/kg) reduces thermal hyperalgesia in arthritic mice through a CB_1 receptor mechanism of action. An acute injection of URB (10 mg/kg, i.p.) reduced the hyperalgesic responses of arthritic wild type mice in (c) the hot plate and (d) tail immersion tests. Pretreatment with rimonabant (Rim; 3 mg/kg, i.p), but not SR144528 (SR2; 3 mg/kg, i.p), completely blocked both antihyperalgesic effects of URB597. Mice were evaluated for nociceptive latencies in both tests before injections and again 1 h after injections. #$p<0.05$ vs. vehicle-treated arthritic and URB+Rim groups; **$p<0.01$; ***$p<0.001$ vs. corresponding pretreatment condition (planned comparison). Data depicted as means ± SEM (n = 6–9 male DBA1/J mice per group).

CB_1 receptor antagonism reverses the anti-hyperalgesic phenotype of prolonged FAAH inhibition in mice via repeated administration of URB597, indicate that multiple cannabinoid receptors are involved. Moreover, these data support the hypothesis that increased anandamide levels play a predominant role in dampening the severity of CIA because it is the only known FAAH substrate that binds to cannabinoid receptors. However, the apparent involvement of anandamide does not preclude the possibility that other substrates of FAAH may also have beneficial effects in the CIA model. In addition to anandamide, FAAH metabolizes a variety of lipid signaling molecules, including *N*-palmitoylethanolamide (PEA), *N*-oleoylethanolamide (OEA), oleamide, and the N-acyl taurines (Cravatt et al., 2001; Saghatelian et al., 2006). Genetic deletion or pharmacological inhibition of FAAH increases levels of each of these lipids in vivo. For example FAAH (−/−) mice possess elevated levels of PEA, and OEA in brain, spinal cord, testis, liver and kidney, as compared with wild type mice. FAAH-NS mice, on the other hand, have elevated PEA and OEA in non-neuronal tissues, but normal levels in nervous tissue (e.g., brain and spinal cord) (Cravatt et al., 2004). PEA has long been known to possess anti-inflammatory actions (Coburn et al., 1954). More recently, the anti-inflammatory and anti-hyperalgesic effects of PEA and OEA in the carrageenan model of inflammatory pain have been shown to be mediated through a PPARα receptor mechanism of action (D'Agostino et al., 2009; D'Agostino et al., 2007; Genovese et al., 2008; Lo Verme et al., 2005; Lo Verme et al., 2004; LoVerme et al., 2006). These findings raise the possibility that, in addition to the cannabinoid effects reported herein, non-cannabinoid FAAs may elicit anti-inflammatory and anti-hyperalgesic effects on their own and may also augment the actions of anandamide. Similarly, elevated FAAs may bind to other targets to attenuate CIA-induced inflammation and thermal hyperalgesia. At high concentrations, anandamide is a ligand for TRPV1 receptors (Di Marzo and De Petrocellis, 2010; Schlosburg et al., 2009b). For example, the TRPV1 receptor antagonist capsazepine blocks the antiedematous and anti-hyperalgesic effects of anandamide in the carageenan model (Horvath et al., 2008).

An attractive feature of FAAH inhibitors is their apparent lack of untoward side effects. URB597 does not produce THC-like effects in the drug discrimination paradigm (Gobbi et al., 2005) and lacks the cannabimimetic activity of cannabinoid receptor agonists (Compton et al., 1992). Unlike Δ^9-tetrahydrocannabinol (THC), the primary active constituent of marijuana (Justinova et al., 2003; Tanda et al., 2000), URB597 is not self-administered by nonhuman primates (Justinova et al., 2008) and does not support conditioned place preferences in rodents (Gobbi et al., 2005; Scherma et al., 2008). Similarly, unlike THC, FAAH inhibition does not cause locomotor suppression (Kinsey et al., 2011b). The lack of reinforcing effects supports the idea that FAAH inhibition lacks abuse potential. Also, unlike the consequences of repeated administration of cannabinoid agonists such as WIN55,212-2 or THC (Aceto et al., 1996; Aceto et al., 2001; Tsou et al., 1995), repeated administration of URB597 does not lead to physical dependence (Schlosburg et al., 2009a). Finally, unlike nonsteroidal anti-inflammatory drugs (NSAIDs), which are well known to cause gastrointestinal ulcers, URB597 produces gastro-protective effects against NSAID-induced ulcers (Naidu et al., 2009).

The present results demonstrate that FAAH inhibition or genetic deletion functions via multiple cannabinoid receptor levels to dampen the development of collagen-induced arthritis and concomitant hyperalgesia. The observation that these phenotypes are maintained

in FAAH-NS mice, in which FAAH is exclusively expressed in the nervous system, suggests that targeting non-neuronal FAAH is sufficient to reduce CIA. These results are consistent with the recent finding that the peripherally restricted FAAH inhibitor, URB937, decreases nociceptive behavior in sciatic nerve ligation, carrageenan, and formalin models of pain (Clapper et al., 2010). Moreover, URB937 reduced the development of carrageenan-induced paw edema. Thus, blocking endocannabinoid metabolism could have the added advantage in the treatment of rheumatoid arthritis by producing dual anti-inflammation and anti-hyperalgesia, without the rewarding effects and abuse potential of direct cannabinoid receptor agonists.

5. Conclusions

Prolonged FAAH blockade in non-neuronal tissue reduces the severity of CIA through a CB_2 receptor mechanism of action. In contrast, CB_1 receptors mediate the reduction of CIA-induced thermal hyperalgesia caused by acute inhibition of FAAH. These findings demonstrate that simultaneous elevations in neuronal and non-neuronal endocannabinoid signaling are possible through inhibition of a single enzymatic target (FAAH), thereby offering a potentially powerful strategy to treat chronic inflammatory pain syndromes that operate at multiple levels of anatomical integration.

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