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Review Article

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Cannabinoids, Endocannabinoids, and Related Analogs in Inflammation

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Abstract. This review covers reports published in the last 5 years on the anti-inflammatory activities of all classes of cannabinoids, including phytocannabinoids such as tetrahydrocannabinol and cannabidiol, synthetic analogs such as ajulemic acid and nabilone, the endogenous cannabinoids anandamide and related compounds, namely, the elmiric acids, and finally, noncannabinoid components of *Cannabis* that show anti-inflammatory action. It is intended to be an update on the topic of the involvement of cannabinoids in the process of inflammation. A possible mechanism for these actions is suggested involving increased production of eicosanoids that promote the resolution of inflammation. This differentiates these cannabinoids from cyclooxygenase-2 inhibitors that suppress the synthesis of eicosanoids that promote the induction of the inflammatory process.

KEY WORDS: ajulemic acid; cannabinoid; elmiric acid; endocannabinoid; inflammation.

INTRODUCTION

This review is intended to be an update on the topic of the involvement of cannabinoids in the process of inflammation. Other reviews cover certain aspects of this subject and the reader is referred to them for a discussion of earlier reports (1–4). In this review are reports published in the last 5 years on the activities of all classes of cannabinoids, including the endogenous cannabinoids such as anandamide, related compounds such as the elmiric acids (EMAs), and noncannabinoid components of *Cannabis* that show anti-inflammatory action. An interesting recently published example of the latter one is caryophyllene, an abundant component of *Cannabis* oil that shows anti-inflammatory activity and has high affinity for cannabinoid receptor 2 (CB2; 5).

CLASSICAL CANNABINOIDS

The term cannabinoid strictly speaking refers to compounds that can activate either the cannabinoid receptor 1 (CB1) or CB2 receptor, or both. However, other molecules that have structures similar to tetrahydrocannabinol (THC; Fig. 1), but do not activate the receptors, have often been included under this term. In addition, a number of *Cannabis* components that also do not activate the receptors are often called cannabinoids. In fact, some 50–60 of these substances have been isolated; however, only a handful have been shown to activate CB1 or CB2.

Phytocannabinoids: Tetrahydrocannabinol and Cannabidiol

The role of THC in lymphocyte biology and immune/inflammatory responses has been reviewed extensively (6–8). Experiments with THC in animal models remain important despite its psychoactivity, to help understand the wider endocannabinoid system and the complex functions of the CB1 and CB2 receptors. In one such model (9), THC reduced airway inflammation in mice. Atherosclerosis, a chronic inflammatory disease, is the primary cause of myocardial infarction and stroke. Administration of THC reduces development of experimental atherosclerosis and its cardiac and cerebral manifestations (10). However, a protective role for CB1 blockade was also observed, reinforcing the complexity of cannabinoid biology.

Cannabidiol (CBD; Fig. 1) is usually the most abundant nonpsychoactive cannabinoid in the plant, and it and analogs of CBD have been studied more extensively in recent years. Thus, CBD reduces joint inflammation in collagen-induced arthritis (CIA) in mice (11) and carrageenan paw edema in rats (12). CBD treatment also suppressed release of tumor necrosis factor (TNF)α from synovial cells isolated from the mice. In addition, oral administration of CBD (2.5–20 mg/kg) reduces neuropathic (sciatic nerve constriction) and inflammatory (intraplantar injection of complete Freund's adjuvant) pain in rats, effects reversed by vanilloid but not CB receptor antagonists (13). Although CBD did not reduce inducible

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ABBREVIATIONS: AJA, ajulemic acid; AG, arachidonoyl glycerol; CBCr, cannabichromene; CBD, cannabidiol; CBN, cannabinol; EC, endocannabinoid; EMA, elmiric acid; FAAH, fatty acid amidohydrolase; PEA, palmitoyl ethanolamide; THC, tetrahydrocannabinol.

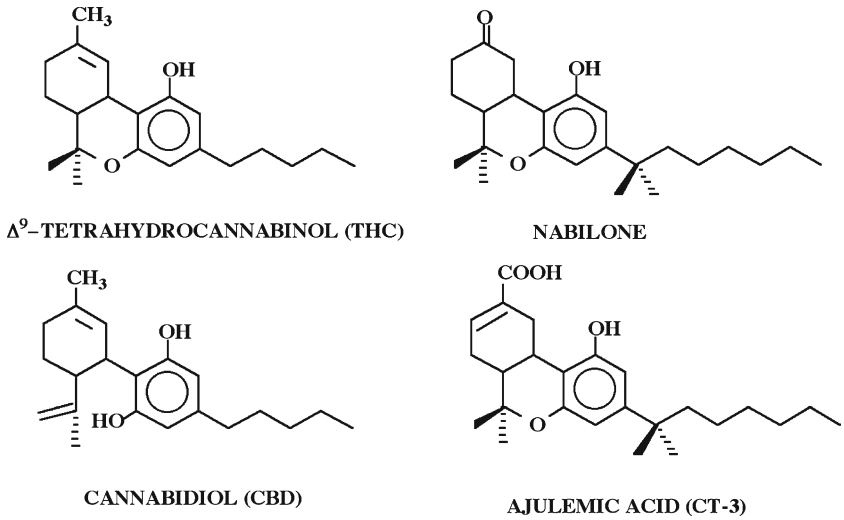

Fig. 1. Phytocannabinoids and analogs. THC and CBD are the principle examples of the 80 odd cannabinoids identified in *Cannabis*. Nabilone (Cesamet®) and ajulemic acid (AJA, CT-3, or IP-751) are synthetic analogs of THC that have shown therapeutic effects in humans

nitric oxide synthase (iNOS) in these studies, others (14,15) have reported that CBD does inhibit iNOS in a beta-amyloid-induced murine model of neuroinflammation. In contrast to these receptor studies, increased activation of rat mast cells by CBD was not mimicked by a full agonist of vanilloid receptor type 1 (16). In addition, CBD is an antagonist of CB receptor agonists in mouse brain and in membranes from cells transfected with human CB2 receptors (17). Binding of CBD and its analogs to the cannabinoid receptors CB1 and CB2 appears to be negligible (18). The dimethylheptyl-7-oic-acid analog of CBD (DMH-CBD) reduces joint inflammation, including cartilage degradation and bone erosion in murine CIA (11). CBD also reduces intestinal inflammation in mice (19). In addition to its ability to suppress production of the inflammatory cytokine TNFα, CBD appears to exert anti-inflammatory activity by suppressing fatty acid amidohydrolase (FAAH) activity, thereby increasing concentrations of the anti-inflammatory endocannabinoid anandamide. CBD and CBD-DMH have been hydrogenated to give four different epimers (20). The complex mechanisms whereby these compounds exert their effects is illustrated by the fact that hydrogenation at different double bonds has different effects on bioactivities, none of which appear dependent on CB1 activation. Further, insight into mechanisms whereby CBD exerts therapeutic effects is provided by experiments which indicate that CBD attenuates inflammation induced by high glucose in diabetic mice (21). Specifically, CBD treatment reduces mitochondrial superoxide, iNOS, nuclear factor kappa B (NF-κB) activation, and transendothelial migration of monocytes. Another potential therapeutic use of CBD may lie in its ability to counter some undesirable effects of THC (sedation, psychotropic effects, tachycardia), thus suggesting that if given together with THC, it may allow higher doses of THC (22). THC and CBD have been administered as an oral mucosal spray to 58 patients with rheumatoid arthritis (23). Treated patients had significant reduction in pain and improvement in sleep compared to patients given placebo.

Ajulemic Acid (AJA, CT3, IP751)

Numerous compounds have been synthesized with the goal of separating psychotropic activity from analgesic/anti-inflammatory action. Among the most promising is 1′,1′-dimethylheptyl-THC-11-oic acid (Fig. 1; ajulemic acid, AJA). The synthesis, chemistry, and biological activity of AJA have been the subjects of several reviews (3,4,24,25). The observation (26) that oral administration of low dose (0.1 mg/kg/day) AJA suppresses joint inflammation and tissue injury in rats with adjuvant arthritis led to further investigation of its mechanisms of action. As reviewed (4), addition of AJA to human cells *in vitro* reduces production of interleukin (IL)-1b but not TNFα, suppresses matrix metalloproteinases (27), and increases apoptosis of human T lymphocytes. More recently, it has been reported (28) that AJA reduces production of IL-6 by human monocyte-derived macrophages. In the adjuvant arthritis model (see above), synovial inflammation did occur in AJA-treated rats. However, inflammation did not progress, and articular cartilage degradation and bone erosion did not occur as it did in rats treated with placebo. The observations suggest that inflammation resolved earlier in AJA-treated animals. Studies of this mechanism resulted in the findings that addition of AJA to human synovial cells *in vitro* increased production of PGJ_2, an eicosanoid that facilitates resolution of inflammation (29). Moreover, addition of AJA to human blood cells and synovial cells *in vitro* and administration of AJA to mice with peritonitis increase production of lipoxin A_4, an eicosanoid which also acts to resolve inflammation (30).

AJA binds weakly to both the CB1 and CB2 receptors (31,32) and questions have been raised about its lack of psychoactivity in humans that are based entirely on certain preclinical studies (33). However, in a rat model of nerve injury-induced and inflammation-induced pain (34), AJA was more effective than the THC analog HU 210 and did not exhibit the psychotropic activity associated with HU210

treatment. More to the point are studies in humans. In doses up to 80 mg/day, AJA does not induce cannabimimetic central nervous system effects in patients with neuropathic pain (35). A possible explanation for this apparent discrepancy may reside in variations in the blood-brain barrier. A brain/plasma ratio of 0.4 (31) was reported in one preclinical study. Thus, in certain cases such as in humans, AJA may be considered to be a "peripheralized" agent.

Although AJA binds to and activates the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR-γ; 36,37), some of the actions of AJA on cells *in vitro* are PPAR-γ independent (27,28). The crystal structure of the AJA binding domain of the γ-isotype of human PPAR was determined at 2.8 Å resolution (38). The binding mode is compatible with other known partial agonists of PPAR-γ, explaining their moderate activation of the receptor, as well as the structural basis for isotype selectivity. The authors believe that the crystal structure also provides clues to the understanding of partial agonism itself.

Osteoclasts are large multinucleated cells formed by fusion of hematopoietic precursors of the monocyte/macrophage lineage. In many inflammatory diseases such as periodontal disease, rheumatoid arthritis, and metastatic cancers, excess osteoclast activity leads to bone resorption (39). Addition of AJA to stimulate precursor mouse macrophages and bone marrow cells in culture suppresses development of multinucleated osteoclasts (osteoclastogenesis) and prevents further osteoclast formation in cultures in which osteoclastogenesis had already begun (40). Thus, reduction of osteoclastogenesis may be an additional mechanism whereby AJA prevents bone erosion in joints of rats with adjuvant arthritis.

Other Synthetic Cannabinoids (Analogs of CBD Are Discussed Above)

Nabilone (Cesamet®; Fig. 1), a dimethylheptyl analog of THC, was approved by the US Food and Drug Administration in 1985, but began marketing in the USA in 2006. It is approved for treatment of chemotherapy-induced nausea and vomiting that has not responded to conventional antiemetics and for anorexia and weight loss in patients with AIDS. Results of a double-blind, randomized, placebo-controlled study of patients with fibromyalgia suggest that nabilone can reduce pain and increase quality of life for these patients (41). After 4 weeks of treatment (0.5 mg daily for 1 week, 0.5 mg twice daily during week 2, 0.5 mg in the morning and 1 mg in the evening during week 3, and 1 mg twice daily during week 4), 15 patients given nabilone experienced significant improvement in pain (visual analog scale), on the Fibromyalgia Impact Questionnaire (FIQ), and the ten-point anxiety scale of the FIQ ($p<0.02$ for all measures *vs.* patients given placebo). The 18 patients given placebo did not experience any change in these measures. Adverse events were not serious, but included dry mouth, vertigo, and ataxia in treated patients. It has been suggested (42) that nabilone and other cannabinoids might be better for treatment of chronic rather than acute pain. Correct dosing will also be important. Indeed, a dose of nabilone of 2 mg three times daily was associated with an increase in pain scores in a trial of postoperative pain (43).

The triaryl bis-sulfone (Sch.336) is a novel synthetic cannabinoid that is selective for the human CB2 receptor. Administration of the compound to animals reduces leukocyte trafficking to areas of inflammation (44). Earlier examples of synthetic cannabinoids include CP55,940 and WIN55,212, both of which are nonselective CB1/CB2 receptor agonists. Both compounds reduce IL-6 and IL-8 production by isolated fibroblast-like synovial cells by a non-CB1/CB2 receptor-mediated mechanism (45) and WIN 55,212 reduces cartilage degradation *in vitro* (46). At an intraperitoneal dose of 0.4 mg/kg, CP55,940 protects guinea pigs against antigen-induced bronchospasm and inflammation. Blockade of either the CB1 or the CB2 receptor attenuated the beneficial effects of CP55,940 treatment (47). These reports highlight the problems in development of CB2 selective antagonists. CB2 has undergone rapid evolution, so that results for ligand binding and efficacy cannot be extrapolated automatically from rodent to human CB2 receptor function (48).

ENDOGENOUS CANNABINOIDS

Groups of naturally occurring members of the eicosanoid super family that can activate cannabinoid receptors and are derivatives of long-chain fatty acids have been referred to as endocannabinoids. They are produced rapidly from lipid precursors and, for example, are released from neurons by neurotransmitter action in a receptor-dependent fashion. Similarly, pro-inflammatory agents can release them from immune system cells. They can then activate cannabinoid receptors on the same or nearby cells and, in some cases, are metabolized rapidly by subsequent enzymic hydrolysis by a specific serine hydrolase called fatty acid amide hydrolase. One of the most important members of the endocannabinoids is anandamide (Fig. 2), the amide conjugate of arachidonic acid and ethanolamine. Other examples are 2-arachidonylglycerol (2-AG; Fig. 2) and the most recent is virodamine. Altogether, there are three enzymes known that have the ability to hydrolyze endocannabinoids, namely, fatty acid amide hydrolase (49–51), monoglyceride lipase, and *N*-acylethanolamine-hydrolyzing acid amidase (52).

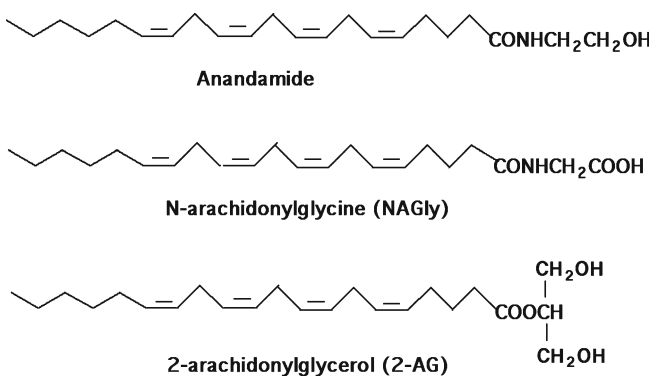

Fig. 2. Chemical structures of endogenous compounds. Anandamide and 2-AG are the two most studied endocannabinoids and each binds to and activates the cannabinoid receptors. *N*-Arachidonyl glycine is the best-studied member of the elmiric acid family of lipoamino acids and does not bind to CB1. Using a proposed nomenclature system (*vide infra*), it would be called EMA-1 (20:4)

Endocannabinoids: Occurrence and Functions

The endocannabinoids appear to be involved in a wide range of regulatory functions throughout the animal kingdom. Some examples are control of sensorimotor and motivational aspects of behavior (53); regulation of fertilized egg implantation (54); hypotensive and bradycardic effects (55); cognition and drug dependence (56); interplay between the endocannabinoid system and the hormone-cytokine array that is involved in the control of human pregnancy (57); sleep-wakefulness cycle, memory formation, locomotor activity, and pain perception (58); modulation of the immune response (59); and control of pain in parallel with endogenous opioids (60). Anandamide and 2-AG mediate many of their actions via either the CB1 or CB2 cannabinoid receptor subtypes. These agonist–receptor interactions result in the activation of G proteins, particularly those of the G(i/o) family (61). They also show interesting variations in tissue levels that are dependent on gender and hormonal cycle (62,63).

Endocannabinoids in Inflammation

There are a number of reports on the effects of the endocannabinoids on various aspects of the inflammatory process (64). For example, anandamide was observed to cause a dose-dependent inhibition of mitogen-induced T and B lymphocyte proliferation (65). Evidence was given that this was due to the induction of cell death by apoptosis. Anandamide-induced apoptosis was also found in human neuroblastoma CHP100 and lymphoma U937 cells (66). Also relevant are the reported effects of endocannabinoids on the immune system (67–71).

A major component of the neurodegenerative disease multiple sclerosis (MS) is inflammation and treatment with cannabinoids has been an active area of investigation in both clinical as well as the preclinical models. Recently, the levels of endocannabinoids (ECs) in samples of cerebrospinal fluid from MS patients were compared with samples from healthy subjects and significant differences were observed (72). In a group of 26 relapsing patients *vs.* 25 healthy controls, increased anandamide concentrations, but not 2-AG, were seen. In the brains of mice with experimental autoimmune encephalomyelitis, a preclinical model of MS, similar results were reported. Peripheral lymphocytes from these patients also had elevated anandamide concentrations suggesting its possible involvement in inflammation. The authors believe that other aspects of MS may also be affected by anandamide. In a separate commentary (73), they point out that endocannabinoid manipulation offers a "unique opportunity to modify neuro degeneration and neuro inflammation".

N-Palmitoyl ethanolamide (PEA) was first isolated and identified almost half a century ago. Two recent articles review the current body of information on this interesting lipid (74,75). The transcription factor, peroxisome proliferator-activated receptor-alpha, has been proposed as the receptor-mediating PEA's anti-inflammatory activity. *N*-Stearoyl ethanolamine like PEA does not activate CB1 and is the subject of a recent paper describing its anti-inflammatory properties (76). Using an *in vivo* animal model of an inflammatory response, namely, IgE-induced cutaneous anaphylaxis, the authors found a significant decrease in the response. Interestingly, the effect was not reduced by CB1 or CB2 antagonists, but was inhibited by capsazepine, a transient receptor potential vanilloid receptor 1 antagonist. They also reported that FAAH is an *in vivo* regulator of the concentrations of *N*-stearoyl ethanolamine. These findings suggest that increasing the levels of saturated fatty acid ethanolamides may result in anti-inflammatory actions.

PEA administration and FAAH blockade resulted in significant augmentation of anti-edema effects in the carrageenan-induced paw edema assay (77). The system used in this study involved a comparison of responses in wild-type and FAAH (−/−) mice where the latter one already exhibits a decreased edema volume. Interestingly, neither anandamide nor oleoylethanolamide was particularly effective in contrast to their actions in other models of inflammation.

Celiac disease is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages and is caused by a reaction to gliadin, a gluten protein found in wheat. The only effective treatment is a life-long gluten-free diet; however, a recent study (78) was done to investigate a possible role for endocannabinoids as markers for the inflammatory phase of this disease. Anandamide and PEA concentrations were significantly elevated (100% and 90%, respectively) in active celiac patients as were those of CB1 receptors. The levels returned to normal after remission with a gluten-free diet. In a companion study using a methorexate-induced rat model, similar results were obtained.

A possible role for ECs following bile duct ligation (BDL) in rats, a model for liver disease, was examined (79). Time dependent increases in 2-AG levels between 2 and 4 weeks postsurgery in various organs and vascular beds were measured. The authors concluded that increased 2-AG may be connected to pathophysiologic changes in liver, heart, lung, and kidney following BDL.

In two models of colonic inflammation, 2,4-dinitrobenzene sulfonic acid (DNBS) and oral administration of dextrane sulfate sodium, the involvement of the CB1 receptor was studied as protection against proinflammatory responses (80). Comparing the effects in CB1-deficient mice (CB1(−/−)) with those in wild-type littermates (CB1(+/+)), a greater degree of inflammation was seen in the receptor-deficient mice. It was also reported that genetic deletion of fatty acid amide hydrolase resulted in protection against DNBS-induced colitis. These findings suggest that manipulation of the ECs could be a useful approach for the treatment of intestinal disease that is characterized by an extreme inflammatory response.

Data have been published suggesting that anandamide has a role in hyperinflammatory reactions in periodontitis (81). CB1 and CB2 are expressed by human gingival fibroblasts and are upregulated during periodontal inflammation. IL-6, IL-8, and monocyte chemoattractant protein-1 induced by *Porphyromonas gingivalis* lipopolysaccharide (LPS) in these cells is reduced by anandamide and this effect can be antagonized by AM251 and SR144528. In addition, LPS-triggered NF-κB activation is blocked by anandamide providing further support for its role in modulating periodontitis.

Evidence has been presented that 2-AG may function as an endogenous inhibitor of cyclooxygenase-2 (COX-2) thereby resulting in a protective effect on neurons that are exposed to harmful insults such as those due to inflammation (82). The mechanism appears to involve COX-2 suppression via the

pertussis toxin-sensitive G protein-coupled CB1 receptor and mitogen-activated protein kinases/nuclear factor-B signaling pathways. These findings may provide a novel approach for the treatment of conditions that result in neurodegeneration.

In an elegant study on the anti-inflammatory and proapoptotic activities of anandamide, it was shown that it can inhibit tumor necrosis factor-α-induced NF-κB activation by direct inhibition of the I κB kinase (83). The NF-κB inhibitory activity of anandamide was independent of CB1 and CB2 activation in TNFα-stimulated 5.1 and A549 cell lines. Structure–activity relationships were examined and it was found that analogs with saturated fatty acyl groups were more active than unsaturated analogs. When the ethanolamide group was replaced with a vanillyl group, a potent inhibition of TNFα-induced NF-κB-dependent transcription was observed.

LIPOAMINO ACIDS (ELMIRIC ACIDS[1]) AND OTHER ANALOGS

The older studies on this topic are mainly concerned with lipoamino acids of bacterial origin. These involve amino acid conjugation with complex and unusual fatty acids and little is known about their function in bacteria. More recently, attention has been given to the lipoamino acids present in mammalian species in part because of their possible relationships to the endocannabinoids. EMA-1 (20:4) is an endogenous substance found in rat brain and other sites that occurs in amounts greater than anandamide (84); however, the origin of EMA-1 (20:4; Fig. 2) *in vivo* is not completely understood.

Prior reports (84,85) suggest that EMA-1 (20:4) might have analgesic and anti-inflammatory properties similar to those reported for anandamide (86,87) but would be inactive in assays for psychotropic action such as the "ring test" (88). The latter one was in agreement with a report showing a lack of affinity by EMA-1 (20:4) for the cannabinoid receptor, CB1 (89). There are no reports on EMA-1 (20:4) binding to or activation of CB2. Likewise, nothing has been reported on interactions between EMA-1 (20:4) and the orphan receptor GPR55 that reacts with atypical cannabinoids (90,91) or lysophosphatidylinositol (92).

Several possible targets for EMA-1 (20:4) and perhaps the other elmiric acids have been described. The action of the glycine transporter GLYT2a is inhibited by EMA-1 (20:4) in a reversible and noncompetitive manner (93). Arachidonic acid, anandamide, and R1-methanandamide showed no effect on glycine transport, whereas *N*-arachidonoyl-L-alanine was active. EMA-1 (20:4) also shows complex effects on glycine receptors being both inhibitory and excitatory with a bell-shaped dose–response curve whose maximum is at 10 μM (94). It has been suggested that EMA-1 (20:4) is an endogenous ligand for the orphan G-protein-coupled receptor GPR18 based on preliminary data (95,96). A second orphan receptor, GPR92, has been identified as a putative target for EMA-1 (20:4; 97). However, its ligand promiscuity and lack of a connection to the process of inflammation make it of less interest to this review than GPR18. Like anandamide, EMA-1 (20:4) is also a substrate for COX-2 giving rise to amino acid conjugates of the prostaglandins (98). Other amino acid conjugates have been found in diverse tissues (63), for example, *N*-arachidonoyl-L-serine has recently been isolated from rat tissues (99) and from bovine brain and reported to have vasodilatory effects in rat mesenteric arteries (100).

An interesting and unusual effect on the immune system was reported for EMA-1 (20:4; 101). They observed that EMA-1 (20:4)-induced inhibition of T cell proliferation was controlled by a CB2 gene polymorphism in cells obtained from human subjects with this condition. This CB2 gene variation is thought to be a risk factor for autoimmune effects, suggesting a possible therapeutic target for the elmiric acids in diseases such as rheumatoid arthritis and multiple sclerosis.

Like anandamide, many of the EMAs cause a profound reduction of cell proliferation when tested at concentrations of 1 and 10 μM (102). The cell type studied, RAW264.7, is frequently used as a model for measuring the effects of potential anti-inflammatory agents. In this report, it was found that a complex relationship appears to exist between structure and activity. This has been confirmed by a subsequent study that extended these preliminary observations in other models (vide infra).

EMA-1 (20:4) showed substantial potency (EC-50=4–7 μM) as an *in vitro* inhibitor of FAAH (84), the enzyme primarily responsible for the degradation of anandamide to arachidonic acid and ethanolamine under physiological conditions. However, it had little effect on anandamide transport or on the vanilloid receptor. This suggests as one possibility that EMA-1 (20:4) may act as an endogenous regulator of tissue anandamide concentrations by virtue of its ability to inhibit FAAH and its presence in a number of tissue sites *in vivo*. FAAH −/− mice have 15-fold increased brain levels of anandamide and show decreased pain responses (103) underscoring the role of FAAH in anandamide activity.

A more recent publication (104) extended the aforementioned observations on the elmiric acids. The inhibition of FAAH activity was studied in preparations from several sources with both EMA-1 (20:4) and several of its amino acid congeners. In the case of EMA-1(20:4), it appears that there may be species differences in inhibitory action. The data indicate that there is an appreciable sensitivity to structure as well as species in the inhibition of FAAH. At this time, it is not proven that this activity of the elmiric acids is responsible for any of their effects *in vivo*.

In a newly reported study (102), data were presented supporting the possibility that members of the EMA family are candidates for drugs to treat various inflammatory conditions. A mechanism-based *in vitro* assay for screening libraries of EMAs for potential anti-inflammatory activity based on their stimulatory action on prostaglandin J_2 levels was developed. It is noteworthy that, in contrast to the nonsteroidal anti-inflammatory drugs (NSAIDS), the EMAs are not COX-2 inhibitors. Thus, it is expected that their side effect profile would be different. In the same study, several of the EMAs showed efficacy *in vivo* in mouse models such as

[1] The elmiric acids. A nomenclature system for the lipoamino acids has been suggested. Using this system, *N*-arachidonylglycine, which structure is shown in Fig. 2, would be written as EMA-1 (20:4). EMA stands for elmiric acid; each amino acid constituent is assigned a number, e.g., 1=glycine, 2=alanine, etc. The identity of the acyl substituent is indicated in parentheses, e.g., (20:4)=arachidonoyl, (16:0)=palmitoyl, etc.

the paw edema, leucocyte migration, and ear edema assays. Some indication of the structure–activity profile was also obtained from these experiments. For example, an important factor seems to be the presence of double bonds in the fatty acid portion of the molecule; however, the structure of the amino acid group can also influence its anti-inflammatory action.

In a recent review (2), a putative mechanism for the anti-inflammatory actions of the EMAs has been proposed. The reader is referred to this article for a detailed discussion of the mechanism that contains the following main points. During the resolution phase of an inflammatory process, activation of the arachidonic acid cascade ultimately results in an elevation of 15-deoxy-delta-12, 14-PGJ_2 (105). This prostaglandin appears to be an important promoter of the resolution phase of inflammation. The introduction of a cannabinoid to the site of inflammation results in a further activation of the cascade probably through the action of a receptor leading to an elevation of 15-deoxy-delta-12, 14-PGJ_2. Thus, cannabinoids and related analogs have the effect of speeding up the resolution of inflammation. This differs from the action of the NSAIDs where an inhibition of proinflammatory eicosanoids appears to be the mechanism and which may produce a greater number of side effects.

A mouse thioglycollate-induced peritonitis model has been used to study the anti-inflammatory actions of several cannabinoid receptor ligands (106). The unpublished study shown in Fig. 3 was done using this model and the results are in good agreement with the putative mechanism mentioned above and with the data reported recently on the anti-inflammatory potential of the elmiric acids (102). The cells consisted primarily of peritoneal macrophages and, at a dose of 5 mg/kg EMA-1 (20:4) given orally, produced a significant *decrease in peritoneal cells* along with *an increase in 15d-PGJ_2*. Thus, it appears that the use of this model could yield interesting and valuable information on the mechanism of action of the EMAs. Mouse peritoneal macrophages have also served as models in earlier studies of cannabinoids (107–110).

Preliminary data suggest that the effect of EMA-1(20:4) in the mouse peritonitis model may be mediated by the peripheral cannabinoid receptor CB2 and not the CB1 receptor that is highly expressed in the brain. The CB2 antagonist SR144528 produced a modest reversal of the anti-inflammatory effect (unpublished data); however, confirmation with a second antagonist such as AM630 is needed. This is underscored by two recent reports showing that SR144528 is ineffective in reducing EMA-1 (20:4)-induced analgesic effects (111,112).

Inflammation and cancer could be considered related processes suggesting that the EMAs may be useful in certain anticancer applications. Thus, it has been observed that a selective inhibition of proliferation of human breast cancer cells (HTB-126) *vs.* normal (HTB-125) cells from the same donor can be elicited by the EMA *N*-palmitoyl tyrosine (113). At a concentrations of 3–10 μM, there was separation of activity in favor of the cancer cells (Fig. 4). This inhibition of proliferation could be prevented by the prior addition of the CB1 antagonist SR141716 suggesting that the effect is mediated by this GPCR. The probable low toxicities of the EMAs would make them useful in long-term maintenance therapy following first-line treatment with more potent agents or treatment with radiation or surgery.

PLANT PREPARATIONS AND NONCANNABINOID CONSTITUENTS OF CANNABIS

Cannabis sativa is a complex botanical, and it is not unlikely that the therapeutic benefits of marijuana are due to some of the more than 60 cannabinoids and 200–250 non-cannabinoid constituents of the plant. One noncannabinoid,

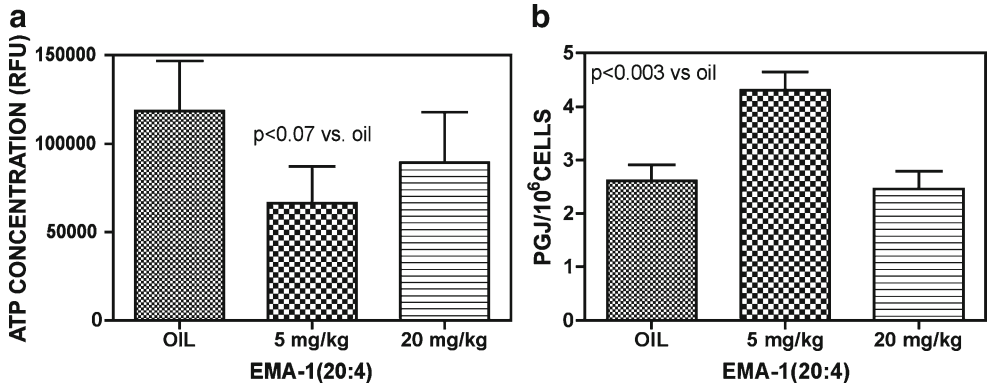

Fig. 3. The *in vivo* effect of EMA-1(20:4) on cell migration (**a**) *vs.* 15d-PGJ_2 production by mouse peritoneal macrophages (**b**). CD-1 male mice 18–20 g body weight were administered the above indicated treatments p.o. and after 30 min, injected i.p. with 1 ml 8% BBL Fluid Thioglycollate Medium. **a** Cells were harvested by peritoneal lavage after 3 h, exposed to lysing buffer for 2 min to remove erythrocytes, resuspended in phosphate buffered saline/bovine serum albumin and an aliquot subjected to the cell TiterGlo assay with four replicates for each sample. **b** The remaining cells were suspended in 1 ml minimum essential medium and plated onto 32 wells of a 48-well dish (eight wells/group). The plates were incubated for 18 h, the media harvested, centrifuged, and assayed for 15d-PGJ_2 by enzyme-linked immunosorbent assay (Assay Designs). Numbers of adherent cells were obtained using the TiterGlo assay that measures ATP levels. The values shown are the prostaglandin concentrations (pg/ml) divided by number of adherent cells

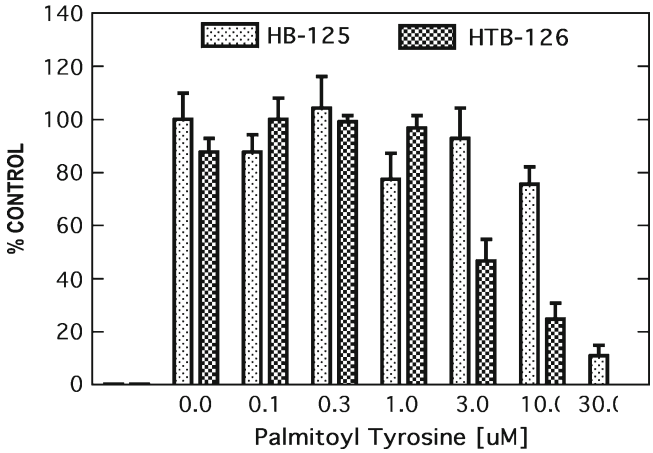

Fig. 4. Inhibition of proliferation of human breast cancer cells (HTB-126) *vs.* normal (HTB-125) cells. HTB-126™ (Hs 578T) cell strain was derived from a carcinoma of the breast. It was originated by Vuong *et al.* (112) along with the Hs 578Bst (HTB-125™), which is a normal fibroblast-like line from the same patient. The donor was a 74-year-old female Caucasian. Hs 578Bst was derived from normal breast tissue peripheral to an infiltrating ductal carcinoma that was the source for HTB-126. The cell proliferation assay, which measures ATP levels, was performed at 48 h following drug treatment and the values are expressed as a percentage of the vehicle-treated wells

the geranylated flavone cannflavin A (Fig. 5), is 30 times more potent than aspirin as an inhibitor of prostaglandin E_2 (114,115). These potentially important findings have been overlooked, as most attention in marijuana research has been directed to the analgesic effects of the plant and to mechanisms of psychoactivity. A further example that this line of inquiry has remained dormant is a series of overlooked observations (116), which demonstrate potent anti-inflammatory actions of a crude marijuana extract and of the nonpsychoactive *Cannabis* constituents, CBD, cannabinol, and cannabichromene in the carrageenan paw edema model of acute inflammation in rats. Volatile oil products of the plant also have biological activity (117). Thus, pyrolysis products may add to the therapeutic properties of smoked marijuana. Several of the most abundant cannabinoid and noncannabinoid constituents of *C. sativa* are nonpsychoactive (118).

As noted, CB2 receptor-selective agonists that are devoid of the psychoactivity associated with CB1 receptor activation are potential drug candidates for treatment of a range of diseases, most notably those associated with pain and inflammation. It is therefore of much interest that *C. sativa* essential oil devoid of the classical cannabinoids displaced a high affinity radioligand from human CB2 but not CB1 receptors. Fractionation of the *Cannabis* essential oil and screening of the isolated constituents yielded (E) β-caryophyllene ((E)-BCP; Fig. 5), which selectively binds to the CP55,940 binding site in the CB2 receptor and inhibits LPS-stimulated TNFα and IL-1b expression in human peripheral blood (5). In addition, oral administration of 5 mg/kg (E)-BCP reduces carrageenan paw edema in mice. That this sesquiterpene is found in large amounts in the essential oils of commonly used spices such as cloves, rosemary, oregano, cinnamon, and black pepper suggests that low dose β-caryophyllene may be available by dietary means.

Flavonoids are ubiquitous plant phenolic compounds that consist of two aromatic rings linked by a three carbon bridge. They are attracting interest because of their antioxidant, antitumor, anti-inflammatory, and antimicrobial activities. The flavone luteolin, a constituent of *C. sativa*, is also found in spices and in vegetables such as celery and green pepper. When added to peripheral blood mononuclear cells *in vitro*, luteolin suppresses production of the inflammatory cytokines TNFα, IL-1b, and IL-6, actions that relate to a selective reduction in numbers of monocytes (119). Perhaps more importantly, luteolin inhibits growth of *Plasmodium falciparum in vitro* (120) and protects against induction of colon cancer in mice (121).

CONCLUSIONS

Possibly the very earliest literature reference on *Cannabis* describes its use as an anti-inflammatory agent. The Chinese emperor Shen-nung (ca. 2000 B.C.), in a work called *Pen-ts'ao Ching*, noted many of the effects of *Cannabis* in humans. Among other properties, it was claimed that cannabis "undoes rheumatism", suggesting possible anti-inflammatory effects (122). The reports described in this review of the current literature provide support for the claims made by the ancient Chinese healers. These more recent publications include relief from chronic neuropathic pain, fibromyalgia, rheumatoid arthritis, and postoperative pain. In addition, a large body of preclinical data on all classes of cannabinoids, including the endogenous examples, point to a variety of therapeutic targets for cannabinoids and important roles for the endocannabinoids in the physiology of inflammation

"MA" (Cannabis)

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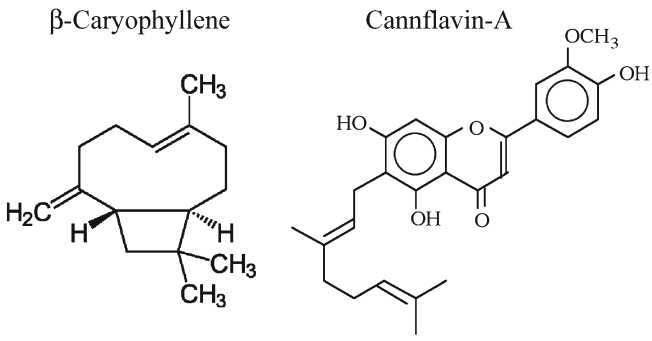

Fig. 5. Structures of noncannabinoid plant constituents

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