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Beyond THC and Endocannabinoids

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Abstract

Research in the cannabinoid field, namely on phytocannabinoids, the endogenous cannabinoids anandamide and 2-arachidonoyl glycerol and their metabolizing and synthetic enzymes, the cannabinoid receptors, and anandamide-like cannabinoid compounds, has expanded tremendously over the last few years. Numerous endocannabinoid-like compounds have been discovered. The Cannabis plant constituent cannabidiol (CBD) was found to exert beneficial effects in many preclinical disease models ranging from epilepsy, cardiovascular disease, inflammation, and autoimmunity to neurodegenerative and kidney diseases and cancer. CBD was recently approved in the United States for the treatment of rare forms of childhood epilepsy. This has triggered the development of many CBD-based products for human use, often with overstated claims regarding their therapeutic effects. In this article, the recently published research on the chemistry and biological effects of plant cannabinoids (specifically CBD), endocannabinoids, certain long-chain fatty acid amides, and the variety of relevant receptors is critically reviewed.

INTRODUCTION

CBD: cannabidiol

THC: Δ^9-tetrahydrocannabinol

CB1/2: cannabinoid 1 or 2 receptor

2-AG: 2-arachidonoyl glycerol

Investigations on cannabinoids can be seen as encompassing three areas of research: (*a*) plant cannabinoids, (*b*) endogenous cannabinoids, and (*c*) endogenous endocannabinoid–like compounds. This review covers some recent advances in these three domains of cannabinoid research and thus is not a comprehensive review.

Research on plant cannabinoids began in the last decades of the nineteenth century, leading to the isolation of cannabinol and cannabidiol (CBD) and the tentative isolation of a tetrahydrocannabinol by the end of the 1940s [for an early review, see Todd (1)]. With the advent of modern techniques, starting in the 1960s, Δ^9-tetrahydrocannabinol (THC) and numerous other plant cannabinoids were isolated, and their structures, including that of CBD, were elucidated. Most were also synthesized, and biological investigations were initiated (2, 3). Advances in the chemistry of the plant cannabinoids and the upsurge of cannabis use in the Western world led to extensive pharmacological and physiological research, particularly on THC and somewhat less on CBD, which continues unabated. Some clinical studies were also reported (for background reviews, see 4, 5). By the mid-1980s, a fairly detailed picture of the pharmacology of plant cannabinoids had emerged, but the mechanism of these effects remained unknown.

As cannabinoids act stereospecifically (6), it was reasonable to assume that THC binds to a pharmacologically relevant cannabinoid receptor (CB). Research by Allyn Howlett and colleagues (7) led to the identification of such a receptor (now known as CB1), and later work by Munro et al. (8) identified a second receptor (CB2). Both receptors are activated by THC, but while the activation of CB1 [predominantly expressed in the central nervous system (CNS)] leads to typical cannabis psychoactivity, the activation of CB2 (primarily expressed in various immune cells) does not and is generally involved in protective biological actions (9). As receptors are presumably formed in the living body to be activated not by plant constituents but rather by endogenous molecules, a search for such molecules was initiated and led to the identification of arachidonoyl ethanolamide (anandamide) (10) and 2-arachidonoyl glycerol (2-AG) (11, 12) as endogenous agonists of these receptors (see **Figure 1**). The enzymes involved in the synthesis and hydrolysis of these agonists were also identified, and thus an endocannabinoid system was established. As several reviews were recently published on endocannabinoid biosynthesis and metabolism (13, 14), these topics are not reviewed in detail here. Today, the endocannabinoid system is known to be involved in a long list of physiological processes/functions and pathological disease states (9, 15).

PLANT CANNABINOIDS AND MAMMALIAN ENDOCANNABINOIDS: RECENT ADVANCES

More than 100 plant cannabinoids, which are terpeno-phenols, have been isolated from *Cannabis sativa* (16). The plant does not produce neutral cannabinoids, which are formed from the respective natural aromatic acids by nonenzymatic decarboxylation. Research on the cannabinoid acids has not received much attention because they are not stable and decarboxylate to the neutral cannabinoids; any results with them are difficult to interpret, as they may be due to either the acids or the newly formed neutral cannabinoids. Recently, it was reported that the methyl ester of cannabidiolic acid is stable and, like CBD, has anti-nausea and antianxiety activity (17) and reduces depression-like behavior in two genetic animal models of depression (18). The question remains as to whether the stable methyl ester parallels the activity of CBD in other areas.

Although thousands of publications have addressed anandamide activities, with slightly fewer on 2-AG activities, and neither compound has shown toxic effects in animals, these endogenous constituents have never been administered to humans. Are we missing something?

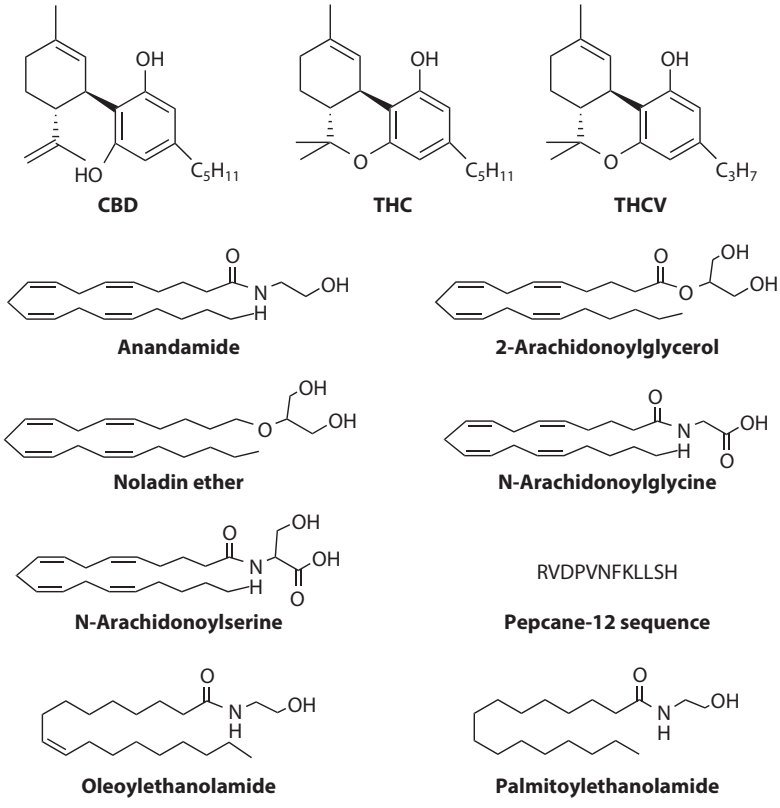

Figure 1

The structures of selected cannabinoids. Abbreviations: CBD, cannabidiol; THC, Δ^9-tetrahydrocannabinol; THCV, tetrahydrocannabivarin.

The minor constituents homo-γ-linolenoyl ethanolamide and docosatetraenoyl ethanolamide were also extracted from porcine brain and were shown to inhibit the specific binding of a labeled cannabinoid to the CB1 receptor, with inhibitory constant values close to those of anandamide (19). However, these constituents have not been biologically investigated to any large extent, whereas many long-chain fatty acid amides with amino acids (NAAAs) (or related entities) have been identified, and some of them are discussed below.

Anandamide is an amide and 2-AG is an ester, and both are chemical moieties that can be affected by amidases and esterases. Anandamide is predominantly hydrolyzed by a specific fatty acid amide hydrolase (FAAH) (20) and 2-AG by a monoacylglyceryl lipase (21). However, a 2-arachidonoyl glyceryl ether named noladin, which was also found in porcine brain (22), is much more stable, as no specific etherase has been found. The existence of noladin as an endogenous substance was initially questioned (23), but recently, Juerg Gertsch of the University of Bern confirmed the presence of noladin ether in the blood of both mice and humans (J. Gertsch, unpublished observations).

NAAA: *N*-acyl amino acid

FAAH: fatty acid amide hydrolase

Because endocannabinoids are lipophilic molecules synthetized in biological membranes (predominantly in postsynaptic membranes in the brain) on demand, they require the presence of cytosolic binding proteins that chaperone these molecules to intracellular targets and that may also be involved in their uptake and transport (e.g., transport of endocannabinoids to the

presynaptic membranes to exert CB1-dependent biological effects and undergo metabolism). Huang et al. (24) have shown that the fatty acid–binding protein (FABP) FABP1 is the most prominent endocannabinoid and cannabinoid-binding protein in the liver (but absent in the brain), and its deletion raises hepatic endocannabinoid levels. Other FABPs are also present in the brain with similar functions (25).

Several endogenous peptides, shown to have CB1 agonist or inverse agonist activity, have been reported (26, 27). A group of peptides named Pepcans were found to cause negative allosteric modulation of CB1 (e.g., Pepcan-12), but their physiological role has not yet been elucidated (28).

TRPV: transient receptor potential cation channel subfamily V

PPAR: peroxisome proliferator–activated receptor

AraS: *N*-arachidonoyl serine

AraG: *N*-arachidonoyl glycine

PEA: *N*-palmitoyl ethanol amide

OEA: *N*-oleoyl ethanol amide

LPA: lysophosphatidic acid

CANNABINOID RECEPTORS

In addition to the main G protein–coupled CB1 and CB2, endocannabinoids may also activate, albeit at higher concentrations, transient receptor potential cation channel subfamily V member 1 (TRPV1) channels (29) and numerous other receptors (30–34), including GPR18, GPR55, GPR92, GPR119, and peroxisome proliferator–activated receptor α (PPARα) (for a recent review, see 35). The pharmacology of GPR18 and GPR55 is related to that of CB1 and CB2, while that of GPR92 and GPR119 differs considerably.

In 2007, the binding of several cannabinoids to the G protein–coupled receptor (GPCR) GPR55 in the brain was described (36, 37). This receptor was also found to be activated by lysophosphatidylinositol (38). The pharmacology of GPR55 activation by endocannabinoids is complex. Some studies suggest that GPR55 is coupled to $G\alpha_{12/13}$ (and probably to $G\alpha_q$) proteins and thus activates the small GTPase RhoA and causes Ca^{2+} mobilization. In contrast, *N*-arachidonoyl serine (AraS) appears to act through GPR55 via $G\alpha_{i/o}$ proteins and the downstream phosphorylation of extracellular signal–regulated kinases (ERKs) and Akt. These pathways have long been associated with cell proliferation and migration, supporting the proposal that GPR55 displays agonist-dependent signaling pathways (39). 2-AG may also act through GPR55 (40).

GPR18 was also proposed to be a novel cannabinoid endothelial receptor. It is activated by *N*-arachidonoyl glycine (AraG), AraS, *N*-palmitoyl ethanol amide (PEA), abnormal CBD, and 2-AG via $G\alpha_{i/o}$ proteins (41, 42). It was recently shown that AraG can also initiate a GPR18-independent activation of BK_{Ca} channels in mice aortic endothelial cells, which might also contribute to the vasodilation attributed to cannabinoids (43).

GPR119 was reported to be a receptor for *N*-oleoyl ethanol amide (OEA) (44). It is also activated by oleoyl- and palmitoyl-lysophosphatidylcholine (45). This receptor is coupled to the adenylyl cyclase–cAMP pathway (46). Oleoyl glycerol, an analog of the endocannabinoid 2-AG, was also found to be a GPR119 ligand (47).

GPR92 is a lysophosphatidic acid (LPA) receptor (48). AraG was later found to be a more potent ligand than LPA for this receptor, thereby activating the $G\alpha_s$ pathway (49).

Another target for endocannabinoids is the PPARα nuclear receptor. OEA binds with high affinity to the purified ligand-binding domain of PPARα, and both OEA and PEA activate PPARα in cell-based assays with EC_{50} values of 120 nM and 3.1 μM, respectively (50, 51).

The pharmacology of cannabinoids is further complicated by the fact that different compounds activate different pathways through the same receptors. Cannabinoid receptors, as most of the GPCRs, are able to bind a number of ligands, each of them stabilizing a number of active receptor conformations, contrary to previous ideas implicating an on-off key-lock approach. Each active conformation displays differential activation for downstream intracellular pathways. This ability of a ligand to differently activate specific signaling pathways is called biased agonism (for reviews of biased agonism on CB1 and CB2, see 52, 53).

While 2-AG and synthetic WIN 55,212-2 show little preference for the inhibition of cAMP and phosphorylated extracellular signal–regulated kinase 1/2 (pERK1/2), anandamide and the synthetic agonists CP55940 and HU-210 are biased toward cAMP inhibition. The allosteric modulator Org27569 displayed biased allosteric effects by blocking cAMP inhibition, while simultaneously having little or no effect on ERK1/2 phosphorylation (53). A similar picture can be seen for CB2, for which marked differences were found in the ability of certain agonists to activate distinct signaling pathways and to cause off-target effects (54). For example, the CB2-biased agonist LY2828360 inhibits cAMP accumulation and activates ERK1/2 signaling while failing to recruit arrestin, activate inositol phosphate signaling, or internalize CB2 (55). When three different cannabinoid ligands, THC, WIN 55212-2, and ACEA, were assayed in mouse brain cortex, it was found that the specific pattern of G protein subunit activation was different depending on the ligand (56). When calcium mobilization and ERK1/2 phosphorylation were quantified in a cell line stably expressing GPR18 (HEK293/GPR18 cells) and in a CHO-K1 GPR18 β-arrestin cell line, GPR18 activation was found to involve several signal transduction pathways indicative of biased agonism, thereby providing a plausible explanation for the apparent discrepancies in GPR18 activation found in the literature (57). Biased agonism at GPR55 may help explain the inconsistencies in GPR55 pharmacology seen with an array of ligands.

Biased agonism is also observed with GPR119. Hassing et al. (58) found the degree of constitutive activity of GPR119 to be 1–10%, 10–30%, and 30–70% of the OEA-induced E_{max} in $G\alpha_i$-, $G\alpha_q$-, and $G\alpha_s$-driven pathways, respectively. This coincided with the lowest and highest OEA potency observed in $G\alpha_i$-, $G\alpha_q$-, and $G\alpha_s$-driven pathways, respectively. The conclusion of the authors, as in previous cases, was that their studies uncovering broad and biased signaling explain why many GPR119 drug-discovery programs have failed so far. Thus, biased signaling has to be taken into account while either looking for the activity of endogenous molecules or preparing synthetic ligands for CBs.

ENTOURAGE EFFECT

About 20 years ago it was reported that the biological activity of 2-AG can be increased by two related endogenous 2-acyl-glycerols, 2-linoleoyl-glycerol and 2-palmitoyl-glycerol, which on their own showed no significant activity in any of the tests employed. 2-linoleoyl-glycerol and 2-palmitoyl-glycerol do not bind to the CBs, nor do they inhibit adenylyl cyclase via CB1 or CB2; however, they potentiate the binding of 2-AG and its apparent capacity to inhibit adenylyl cyclase. Together these esters also potentiate 2-AG inhibition of motor behavior, immobility on a ring, analgesia on a hot plate, and hypothermia caused by 2-AG in mice. 2-linoleoyl-glycerol, but not 2-palmitoyl-glycerol, inhibits the inactivation of 2-AG by neuronal and basophilic cells. These observations suggest that inactive 2-acyl-glycerols enhance 2-AG activity by inhibiting its inactivation and possibly via other as-yet-unknown mechanisms. We suggested that this entourage effect may represent a novel route for molecular regulation of endogenous cannabinoid activity (59).

The biochemical basis of some entourage effects has been clarified in some reactions. De Petrocellis et al. (60) found that long-term treatment of human breast cancer cells with PEA downregulates the expression of FAAH, the enzyme responsible for anandamide degradation, thereby leading to an enhancement of anandamide-induced and CB1-mediated cytostatic effects on these cells.

As noted earlier, anandamide is also a full agonist of TRPV1. PEA enhances the TRPV1-mediated effects of anandamide and capsaicin on Ca^{2+} influx into cells. These entourage effects of PEA might be attributable to modulation of TRPV1 activity and could underlie the enhancement of the antiproliferative effects of TRPV1 receptor agonists by PEA.

NAE: *N*-acyl ethanol amide

Garcia et al. (61) have shown that PEA acts as an entourage compound in the spinal cord for the hypotensive effects of intrathecally administered endocannabinoids. Hiley & Hoi (62) have shown that oleamide has cannabinoid-like actions due to its potentiation of the effects of endocannabinoids by inhibiting FAAH-mediated hydrolysis. Smart et al. (63) have investigated a series of saturated *N*-acyl ethanolamides and related compounds and their ability to cause anandamide to produce a Ca^{2+} influx into human embryonic kidney cells expressing the human vanilloid receptor TRPV1. The C12:0, C16:0, C17:0, C18:0, and C18:1 fatty acid ethanolamides greatly potentiated the response to anandamide. De Petrocellis et al. (64) found that while *N*-palmitoyl- and *N*-stearoyl-dopamine are inactive on TRPVR1, they enhance the action on it by *N*-arachidonoyl-dopamine, which they consider an entourage effect. The same group has also shown that PEA enhances the TRPV1-mediated effects of anandamide and capsaicin on Ca^{2+} influx into cells. These entourage effects of PEA might be attributable to the modulation of VR1 activity and could underlie the enhancement of the antiproliferative effects of VR1 receptor agonists by PEA. Russo (65) has reviewed the entourage effects of terpenoids on cannabinoids. It seems possible that these effects are, in part, the reason why some patients prefer cannabinoid extracts rather than pure THC.

In contrast to the above reports, when testing the effects of 2-linoleoyl-glycerol, 2-oleoyl glycerol, and 2-palmitoyl-glycerol on 2-AG neuronal and cell-based signaling assays, Murataeva et al. (66) found that these compounds do not serve as entourage compounds. None of the compounds inhibited neurotransmission via CB1 in neurons, and all failed to potentiate 2-AG-mediated, depolarization-induced suppression of excitation, behaving instead as antagonists. In tests of pERK, cAMP, and arrestin recruitment, none of the acyl-glycerols altered CB1 signaling. As pointed out by the authors, the results show that the relationship between 2-AG and its congeners is more nuanced than previously assumed. We agree.

LONG-CHAIN FATTY ACID AMIDES WITH AMINO ACIDS AND RELATED AMIDES

Although a few NAAAs and long-chain fatty acid amides with ethanol amine (NAEs) were known prior to the discovery of anandamide (10), its discovery led to an increased interest in these types of compounds. Most of the early work was on PEA, which was identified in soybeans, peanuts, and egg yolk and found to be anti-inflammatory (67). Another NAE, OEA, is a satiety factor (68, 69), and in a recent clinical study, OEA was shown to improve inflammation and oxidative stress in obese people (70). OEA was also found to be active in the treatment of alcohol addiction (71) and is involved in addiction to nicotine (72) and cocaine (73). Its mechanism of action is mainly through PPARα (74), TRPV1 channels (75), and GPR119 (46, 76).

More recently, a research group at Indiana University identified 50 novel endogenous acyl amides of amino acids present at 0.2–69 pmol/g in wet rat brain (77, 78); today, 70 such acyl amides are known (79). The variety of amides of NAAAs and NAEs is very large and also includes *N*-acyl ethanolamides, taurine amides, and amides with neurotransmitters, among others. The acyl amides with amino acids and ethanolamines are the most widely investigated compounds of this type. Several reviews have been published recently on the NAEs (80, 81), and although they are not reviewed in detail here, some are discussed below, emphasizing mostly recent publications and those endogenous constituents whose biological activity may have physiological relevance.

The biological effects of the molecules within any of the above groups are not identical. It is plausible that the existence of a biosynthetic mechanism leading to a certain type of compound may be used by cells to generate a variety of related compounds, which may have different mechanisms of action and different activities.

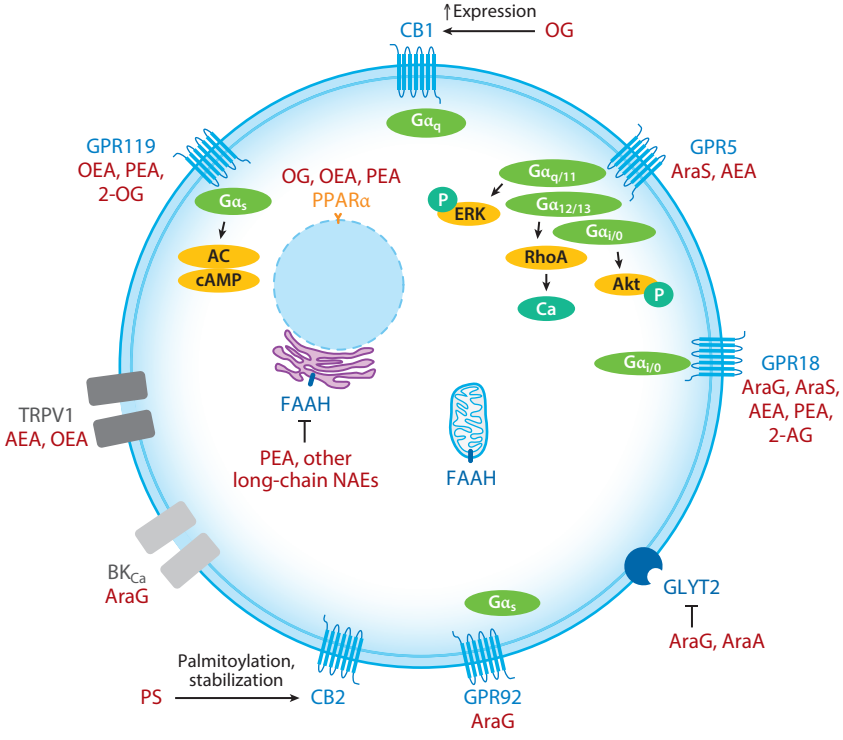

Figure 2

The selected pharmacological targets of the activity of endocannabinoid-like compounds. Endocannabinoids and endocannabinoid-like compounds act on numerous receptors in addition to CB1 and CB2, including TRPV1 channels, BK_{Ca} channels, GPR-family receptors (GPR18, GPR55, GPR119, GPR92), and PPARα nuclear receptors. The pharmacological targets also include activation of ERK, Akt, and RhoA pathways.

Several NAEs inhibit anandamide metabolism by blocking the action of FAAH (82–84). To inhibit the metabolism of anandamide, the fatty acid of NAEs must be longer than a 10-carbon-long chain; shorter NAEs have only a minimal effect (83). Part of the anandamide-potentiating effect of these compounds is due to FAAH inhibition and also possibly to allosteric effects on CBs and vanilloid receptors (63). The potential pharmacological targets of the activity of endocannabinoid-like compounds are shown in **Figure 2**.

Long-Chain *N*-Acyl Amides with Amino Acids: A Few Examples

The biological properties of most of the NAAAs are not yet known. Some of the NAAAs (e.g., *N*-arachidonoyl-glycine, AraS) activate GPCRs.

Long et al. (85) recently identified an enzyme, PM20D1, that produces NAAAs by condensation of fatty acids and amino acids; it also catalyzes the reverse hydrolytic reaction. Mice with increased PM20D1 have increased NAAAs in blood. The authors noted that administration of NAAAs to mice blunted high-fat diet–induced weight gain. Direct intraperitoneal administration of NAAAs to mice increased whole-body energy expenditure, with weight loss and improved glucose homeostasis. The authors suggest that this pathway might be useful for treating obesity and associated disorders.

N-Oleoyl Glycine

OG: *N*-oleoyl glycine

CPP: conditional place preference

Until recently, addiction was assumed to be mainly a psychological state. Now it is believed that addiction represents a CNS disease (86) (for a review of endocannabinoid signaling in reward and addiction, see 87).

If an animal recognizes addiction as an undesirable state (or a disease), it is plausible that the animal may try to lower the effects of such a disease. It is well established that some individuals using addictive drugs do not get addicted, whereas others do. The reason for this difference is not yet known.

Donvito et al. (88) reported investigations aimed at discovering a natural antiaddiction defense mechanism. Their research was based on an observation made by Naqvi et al. (89), who reported that cigarette smokers suffering from traumatic brain injury that included damage to the insula cortex abruptly ceased their nicotine addiction. This observation, along with more recent data (89, 90), showed that the insula may control processes that moderate or inhibit addictive behavior. Donvito et al. (88) postulated the existence of a neurochemical sensitive to brain injury that might counteract nicotine reward and dependence. The authors found that after trauma the mouse insula produces *N*-oleoyl glycine (OG), which has powerful anti–nicotine addiction properties. In mice, OG blocked the establishment of nicotine conditional place preference (CPP), an addiction assay, and reduced withdrawal responses in nicotine-dependent mice. In morphine-dependent rats, OG reduced withdrawal responses but did not affect morphine CPP, demonstrating selectivity. OG activated PPARα in vitro, and a PPARα antagonist restored nicotine CPP in OG-treated mice (88). OG does not interact with the endogenous cannabinoid system.

Wang et al. (91) showed that OG increased the expression of CB1, but not of CB2, promoted adipogenesis, and enhanced the insulin-mediated Akt signaling pathway. The authors suggested a potential role for OG in increasing insulin sensitivity and suppressing obesity and diabetes.

N-Arachidonoyl Glycine

A recent review summarized the numerous effects of AraG (92). AraG is a metabolite of anandamide formed by both oxidative metabolism of the ethanolamine moiety of anandamide and the conjugation of glycine to arachidonic acid, which is released during anandamide hydrolysis by FAAH (93). AraG is best known for its effects on pain (94, 95) and cell migration (42, 96).

AraG is a natural ligand for GPR18, the putative abnormal CBD receptor (41, 42), and an endogenous inhibitor of GLYT2, a glycine transporter (97). It is also a recruiter of BV-2 microglia, hence its effects may lead to anti-inflammatory actions in the brain (42).

While AraG does not activate CBs, it modulates vascular tone. AraG acts as a vasorelaxant, predominantly via activation of Ca^{2+}-activated K^+ channels in small mesenteric arteries in rats. AraG-induced relaxation is attenuated by a nitric oxide synthase inhibitor (98).

N-Arachidonoyl Serine

Brain extracts have been noted to cause vasodilation, and Milman et al. (99) isolated a bovine brain constituent that caused this effect. Its structure was elucidated as AraS. Unlike anandamide, the chemically closely related AraS binds weakly to CB1, CB2, and TRPV1 receptors. It causes endothelium-dependent vasodilation and enhances phosphorylation of p44/42 mitogen-activated protein kinase and protein kinase B/Akt in endothelial cells. AraS also suppresses lipopolysaccharide-induced formation of tumor necrosis factor α (TNFα) in a murine macrophage cell line and in mice.

AraS is neuroprotective after traumatic brain injury, reducing apoptosis and possessing proneurogenic properties (100). These effects were accompanied by a reduction in lesion volume and an

improvement in neurobehavioral function. AraS increased proliferation of neural progenitor cells in vitro and maintained them in an undifferentiated state in vitro and in vivo. Even when AraS was administered 7 days postinjury, significant neuroprotective effects leading to an improvement in neurobehavioral functions were observed.

OS: *N*-oleoyl serine

AraS was also found to induce endothelial cell proliferation, migration, and angiogenesis in vitro. The proangiogenic action is mediated, at least in part, by activation of the GPR55 receptor. AraS, via GPR55, increases phosphorylation of ERKs and Akt and is involved in vascular endothelial growth factor signaling (39).

N-Oleoyl Serine

Osteoporosis, a prevalent degenerative disease, is the result of an imbalance in bone remodeling (101). The incidence of osteoporosis is lower in Greece, which has been attributed to high olive oil consumption (102). Smoum et al. (103) investigated whether an oleic acid derivative (or metabolite) could be involved in this effect. They reported the presence of NAAAs and NAEs in mouse bone. *N*-oleoyl serine (OS) was noted to have potent activity in an osteoblast proliferation assay. In these cells, OS triggers a Gi protein–coupled receptor and ERK1/2. It is not clear which GPCR is involved. In intact mice, OS moderately increases bone volume density, mainly by inhibiting bone resorption. However, in an in vivo mouse ovariectomy model for osteoporosis, OS prevents bone loss by increasing bone formation and markedly restraining bone resorption. The differential effect of exogenous OS in the ovariectomy versus intact animals may be a result of a decrease in skeletal OS levels. These data show that OS is a previously unexplored lipid regulator of bone remodeling.

N-Palmitoyl Serine

As with anandamide and AraS, *N*-palmitoyl serine treatment also improved the neurobehavioral outcome of mice after traumatic brain injury but apparently by a different mechanism. Treatment with *N*-palmitoyl serine did not affect edema and lesion volume. Its neuroprotective action is mediated by indirect activation of the CBs following brain injury. Mann et al. (104) suggested that the mechanism may involve receptor palmitoylation, which reportedly results in the stabilization of receptors and an increase in their activity.

CANNABIDIOL

Here we briefly summarize and discuss the beneficial actions of CBD, based primarily on in vivo studies and available clinical evidence, and briefly mention other constituents of *C. sativa* with efficacy in preclinical disease models. Readers are often referred to recent overviews on specific subjects rather than the original papers.

CBD is one of the most interesting major constituents of marijuana, and its structure was elucidated in 1963 (105). It does not stimulate CB1 and has no psychoactive properties like those of THC. Paradoxically, CBD was long considered to be without significant biological activity despite scattered literature on its potential benefits in seizures (106–108).

A study by Julius Axelrod and his group (109) in 1998 showed that both THC and CBD were more potent neuroprotective antioxidants than the reference antioxidants they used. Subsequently, numerous preclinical reports demonstrated tissue protective and anti-inflammatory effects of CBD in models of neurodegeneration/neuroinflammation, stroke (110), colitis (111), liver (112, 113), kidney injury (114), cardiovascular disease [cardiomyopathies (115, 116), myocarditis

(117), and myocardial infarction (118)], arthritis (119), sepsis (120), primary diabetes (121) and diabetic complications (122, 123), graft-versus-host disease (GVHD) (124), cancer (125, 126), and epilepsy (106, 127–130), among others (122, 131).

MS: multiple sclerosis

Nevertheless, even with over 2,300 publications in PubMed, little has been done to translate these preclinical observations to clinical trials or practice. A turning point came with rare forms of childhood epilepsy almost four decades after the publication of the first positive small-scale clinical observations on the potential antiepileptic effects of CBD in humans (106). The recent success of two double-blind, placebo-controlled, phase 3 clinical trials investigating the effects of pure CBD in rare debilitating genetic forms of epilepsy in children (127–130) led to the US Food and Drug Administration (FDA) approval in 2018 of an oral solution of Epidiolex (CBD) for the treatment of Lennox-Gastaut syndrome and Dravet syndrome in patients two years of age and older. This FDA approval of Epidiolex has also triggered development of numerous CBD-based pharmaceutical products for clinical studies. However, whether CBD is indeed effective in other forms of epilepsy or any other human diseases remains to be seen in future clinical studies.

CBD is also a constituent of the oral mucosal spray Sativex (nabiximols in the United States) (contains 2.7 mg THC and 2.5 mg CBD per dose), which is approved in over 30 countries for the management of pain primarily associated with multiple sclerosis (MS). THC and its synthetic analog nabilone are FDA-approved only for the treatment of chemotherapy-induced nausea and vomiting and to stimulate appetite in cachexia associated with AIDS or terminal tumors (132).

The earliest medicinal use of cannabis described in historical documents was to treat pain (133). Later studies attributed a predominant role for CB1 in the complex antinociceptive effects involving actions in the peripheral sensory nerves, spinal cord, and CNS (132, 134, 135). A potential antinociceptive role of CB2 has also been suggested (136), particularly in inflammatory pain; however, the exact mechanisms and cellular targets are still poorly understood for this receptor (137). Since THC is a partial agonist of both rodent and human CB1 and CB2 (132), based on preclinical data, it was reasonable to expect comparable antinociceptive effects in humans. However, the clinical trials with THC, CBD, and their combinations have painted a different and more disappointing scenario (138, 139). The initial studies primarily focused on their safety/efficacy and symptom relief (e.g., limb spasticity, pain and sleep quality, bladder incontinence) in MS or other pain-related conditions. In three studies, Sativex/nabiximols improved urinary incontinence in MS patients (138). In contrast, assessment of objective outcomes using the Ashworth Scale in other trials evaluating the efficacy of cannabis/Sativex on spasticity in MS found that, at doses lacking overt psychoactivity, these drugs were minimally or not effective. However, by evaluating the subjective, patient-assessed end points (pain, spasms, spasticity, and sleep quality), the treatment was found to be effective. Significant benefits of Sativex/nabiximols compared to placebo were also observed in follow-up studies using a patient-assessed numeric rating scale for spasticity (138). Although some of the benefits observed could be due to mood improvement, this would not explain why the symptoms (spasticity, pain, and sleep quality) were improved in only some of the Sativex/nabiximols-treated participants. In patients treated with THC for one year, improvements using the Ashworth Scale were also noted (138).

The rationale for combining THC and CBD and selecting the ratio present in Sativex in clinical studies was based on empirical evidence suggesting that CBD may attenuate the undesirable psychoactive effects of THC (140). A recent study demonstrating that CBD is a negative allosteric modulator of CB1 provided a possible explanation for this clinical observation (141). A meta-analysis of the data in 34 trials with various combinations of CBD with THC or CBD alone has been reported (140). Of these trials, 16 were conducted in healthy subjects, and the rest were conducted in diverse clinical populations, including subjects with schizophrenia and bipolar mania, MS, neuropathic and cancer pain, social anxiety disorder, cancer-related anorexia, Huntington's

disease (HD), insomnia, and epilepsy. Depending on the study and the THC:CBD ratio, CBD was found to either prolong/intensify or inhibit THC-induced effects (140).

Multiple recent studies also evaluated the therapeutic potential of nabiximols for cancer pain resistant to opioids. Although the adjuvant used was safe and effective in three trials for cancer pain (142–144), the drug disappointingly failed to reach the primary end point or show efficacy in a phase 3 clinical trial. Notably, post hoc analyses revealed that patients who received lower doses of opioids experienced significant benefits from nabiximols on multiple secondary end points, suggesting that nabiximols might have utility in patients with advanced cancer who receive a lower opioid dose, such as individuals with early intolerance to opioid therapy (139).

In most of these studies, Sativex/nabiximols caused generally mild to moderate side effects. Pharmacokinetic characteristics such as first-pass effects in the liver and slow absorption via the oral route can explain the unfavorable efficacy observed in some clinical reports, which may also limit the utility of self-titration (138).

Collectively, in most MS studies, improvements in subjective rather than quantitative symptomatic outcome measures (including pain) were observed with Sativex/nabiximols, which is in agreement with the conclusion of a systematic review by the American Academy of Neurology suggesting that Sativex/nabiximols was "probably effective" for spasticity, pain, and urinary dysfunction (145), supporting the beneficial effects of CBD+THC-based medicines in pain.

Cardiovascular side effects are the most common cause of failure of numerous lead compounds in clinical development, which resulted in termination of trials for pain with peripherally restricted CB1 agonists (146). It is also well documented in both preclinical and clinical studies that THC, particularly in high doses, may induce CB1-dependent cardiovascular adverse effects (146). In contrast to THC, CBD is devoid of cardiovascular side effects and toxicities, even at high doses used for a prolonged period of time, in both rodents and humans (106, 115–117, 127–129). In addition to not having adverse cardiovascular effects, CBD has been reported to attenuate damage in preclinical models of cardiovascular disease/injury, including stroke, myocardial infarction, autoimmune myocarditis, and doxorubicin- and diabetes-induced cardiomyopathies (146).

Cardiovascular Effects: Myocardial Infarction, Cardiomyopathies, Myocarditis, and Stroke

CBD decreased myocardial necrosis in rat and rabbit models of myocardial infarction (118, 147) by attenuating oxidative stress and improving antioxidant defense mechanisms. In mouse and rat cardiomyopathy models induced by the widely used chemotherapeutic drug doxorubicin (known for its cardiotoxicity in humans), CBD improved cardiac function and attenuated the apoptosis of cardiomyocytes and endothelial cells by decreasing oxidative and nitrative stress (116, 148). CBD also corrected the doxorubicin-induced impairment of mitochondrial biogenesis and function in hearts (116). Chronic CBD treatment improved type I diabetes–induced myocardial dysfunction and cell death in mouse hearts and in human cardiomyocytes exposed to high glucose by attenuating NFκB activation and interrelated signaling pathways [e.g., inducible nitric oxide synthase (iNOS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and TNFα expression], decreasing reactive oxygen and nitrogen species (ROS, RNS) generation, cell death, and myocardial fibrosis (115). CBD in primary coronary endothelial cells attenuated the high glucose–induced endothelial cell inflammatory response and barrier disruption (149) and improved diabetic retinopathy (150).

In a model of chronic autoimmune myocarditis in mice, CBD treatment improved cardiac dysfunction, decreased the $CD3^+$ and $CD4^+$ cell–mediated inflammatory response and injury, and modulated myocardial fibrosis (117). These studies provided the rationale for a recent

exploratory clinical trial to assess the safety of CBD in patients with heart failure ACC/AHA stages A-C (NCT03634189).

CBD attenuated cerebral ischemia–induced brain injury in several animal models of stroke (151–153). In most of these studies, the protective effects of CBD on neuronal cell death and the recovery of cognitive function following the insult were independent from CB1/2, which could be attributed to the attenuation of oxidative stress and excitotoxicity, decreased microglial activation and leukocyte infiltration, attenuation of the release of danger-associated molecular patterns from necrotic cells, and improved microcirculation and brain metabolism. In some of the studies, the observed beneficial effects involved 5-HT1A, A2A, and CB2 (153, 154).

Liver and Kidney Injury and Disease

CBD reduced hepatic ischemia reperfusion and alcohol binge–induced liver injury in rodents (112) and the hepatotoxicity of cocaine (155) and cadmium (156). It also improved cognitive function in a fulminant hepatic failure–induced model of hepatic encephalopathy in mice (157). The protective effects in these models involved attenuation of the proinflammatory response, signaling, and cell death. CBD also attenuated human liver sinusoidal endothelial cell activation, attachment of human neutrophils to the activated endothelium, and Kupffer cell activation (112).

CBD increased lipid mobilization and inhibited the development of steatohepatitis in zebrafish and obese mouse models (113). In a chronic plus binge alcohol–induced steatohepatitis model in mice, CBD attenuated alcohol-induced inflammation involving E-selectin expression and neutrophil recruitment and consequent oxidative/nitrative stress. It also attenuated alcohol-induced hepatic metabolic dysregulation in mice and steatosis and oxidative burst in human neutrophils (158). CBD also decreased kidney injury induced by cisplatin or ischemia/reperfusion in mice/rats (114, 159) by decreasing oxidative/nitrative stress and inflammation.

Neurodegenerative Disease, Pain, Epilepsy, Anxiety, Sleep, and Emesis

Oxidative and nitrative stress and inflammation have been implicated in the pathology of most neurodegenerative diseases, including MS, Parkinson's disease (PD), Alzheimer's disease (AD), and HD (160). The beneficial effects of CBD observed in preclinical models of MS (161–163), PD, AD, and HD could be attributed to attenuation of oxidative/nitrative stress, excitotoxicity, microglial activation, inflammatory cell infiltration, inhibition of nuclear factor kappa B (NFkB)-related signaling (e.g., iNOS, COX2, NADPH oxidase expression), and enhancement of antioxidant defense mechanisms independent from direct effects on CBs, potentially involving 5-HT1A and inhibition of adenosine uptake (reviewed in 132, 153, 154).

As described above, CBD inhibits various seizures in both experimental animals and humans. However, despite its FDA approval for the treatment of rare forms of epilepsy, the exact mechanism of its antiepileptic effect in vivo is still elusive; it may involve effects on the 5-HT1 receptor, certain ion channels, and indirect effects on GABA. Anxiolytic-like (164) and antiemetic (165) properties of CBD in different animal models were also described, suggesting the potential involvement of 5-HT1A receptors in these effects (154).

Inflammation, Autoimmunity (Primary Diabetes, Arthritis, Colitis, Sepsis, Autoimmune Encephalomyelitis, and Myocarditis), and Graft-Versus-Host Disease

As mentioned above, anti-inflammatory and tissue-protective effects of CBD have been demonstrated in various inflammatory disease models. In most models, CBD attenuated T cell

infiltration/proliferation, microglial activation, and consequent oxidative stress and inflammatory response.

Based on promising preclinical data, a randomized proof-of-concept study in patients with ulcerative colitis was undertaken with a CBD-rich botanical extract. The primary end point was not reached; however, the authors concluded that a CBD-rich botanical extract may be beneficial for symptomatic treatment of ulcerative colitis (166).

An exploratory phase 2 study found that the combination of CBD with standard GVHD prophylaxis is a safe and promising strategy to reduce the incidence of acute GVHD following allogeneic hematopoietic cell transplantation (124); however, further studies are required to demonstrate efficacy of CBD in GVHD in humans.

Cancer

CBD at relatively high micromolar concentrations was reported to inhibit the proliferation of various human cancer cell lines (e.g., human prostate, breast, and colorectal/gastric carcinoma) and/or promote apoptosis in these cell lines, presumably by increased ROS generation and effects on CB2, TRPV1, TRPM8, COX2, or PPARα. CBD also inhibited cancer cell invasion and metastasis and attenuated the growth of certain solid tumors in mice (reviewed in 154).

Based on preclinical studies demonstrating synergistic benefits of combinations of THC, CBD, and the chemotherapeutic drug temozolomide on glioma cells (167), a phase 2 randomized, controlled, small-scale trial of 21 patients with recurrent glioblastoma investigated the potential benefits of Sativex/nabiximols versus placebo in combination with temozolomide. The preliminary results demonstrated increased one-year survival (83% versus 53%). Moreover, in a recent clinical study, the effect of pharmaceutical-grade synthetic CBD on a range of cancer patients was analyzed over a four-year period. Clinical responses were seen in 92% of the 119 cases with solid tumors, including a reduction in circulating tumor cells in many cases and a reduction in tumor size in other cases, as shown by repeat scans. No adverse effects were observed (168). Despite these encouraging preliminary data, no double-blind, placebo-controlled study has been undertaken to demonstrate efficacy of CBD in any human cancers.

Tetrahydrocannabivarin is another interesting constituent of marijuana that was reported to be a CB1 antagonist at a low (but a CB1 agonist at a high) dose as well as a CB2 partial agonist. Tetrahydrocannabivarin had beneficial metabolic effects in preclinical disease models of obesity and steatohepatitis (113, 169) and was safe in a phase 2 clinical study (170, 171); however, its efficacy in any human disease remains to be demonstrated.

In summary, we have briefly reviewed the therapeutic effects of CBD, primarily focusing on in vivo experimental studies and clinical reports. The therapeutic effects of CBD appear to share very similar fundamental mechanisms across diverse pathologies of multiple organ systems in preclinical disease models (see **Figure 3**). These effects include (*a*) attenuation of oxidative and nitrative stress, (*b*) enhancement of antioxidant defense of parenchymal cells, (*c*) attenuation of cell necrosis and the consequent release of danger-associated molecular patterns from dying cells, (*d*) a decrease in endothelial cell activation and the influx of inflammatory cells and their attachment to the activated endothelium (depending on pathological model, neutrophils, macrophages, T cells, microglia, etc.), (*e*) attenuation of the activation of immune cells, and (*f*) improvement of microcirculation and organ function. In most pathological scenarios, these beneficial effects appear to involve the inhibition of ROS-dependent, NFkB-related gene expression (e.g., iNOS, COX2, ROS-generating NADPH oxidases, TNFα) and stress signaling pathways (e.g., p38, JNK), among others.

The beneficial effects of CBD in preclinical disease models, its proven safety in human clinical trials, and its current FDA approval for the treatment of rare forms of epilepsy all indicate that

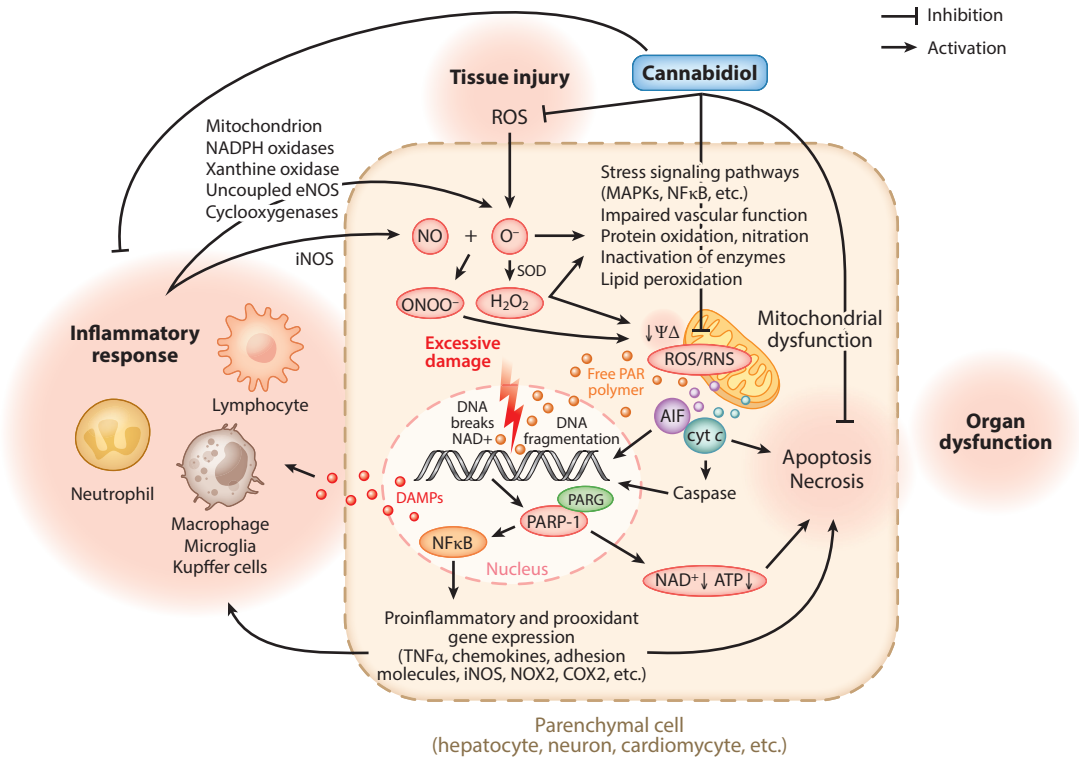

Figure 3

Mechanisms of tissue-protective effects of CBD. CBD in vivo protects against tissue injury and organ dysfunction by attenuating ROS/RNS generation, mitochondrial dysfunction, activation of ROS-dependent stress signaling pathways leading to cell death or proinflammatory response (e.g., various MAPKs, NFκB-dependent mechanisms), and secondary inflammatory response (e.g., chemotaxis, endothelial activation and adhesion of inflammatory cells to activated endothelium, transmigration and activation of immune cells, and consequent inflammatory response and ROS/RNS generation). Abbreviations: AIF, apoptosis-inducing factor; CBD, cannabidiol; COX2, cyclooxygenase 2; DAMP, damage associated molecular pattern; iNOS, inducible nitric oxide synthase; NFκB, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NOX2, ROS-generating NADPH oxidase 2 isoform; O, superoxide; ONOO, peroxynitrite; PARP, poly(ADP)ribose polymerase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase.

its therapeutic potential will be further explored in clinical trials in multiple diseases affecting humans.

In addition to CBD and THC, there are numerous other minor cannabinoids present in cannabis with potential exploitable biological effects for therapeutic gain (172), the discussion of which is beyond the scope of this review.

CONCLUSION

Research in the cannabinoid field involves three distinct areas: the phytochemical, the endogenous cannabinoid (anandamide and 2-AG) and their main CB1/2, and the anandamide-like cannabinoid. Such research has expanded tremendously over the last few years, and new knowledge from all three domains will not only shed light on a large number of physiological processes but may also serve as a starting point for the development of new drugs. It is also tempting to speculate that the large cluster of anandamide-type compounds in the brain, many with CNS effects, is related

to the chemistry of human individual temperamental differences, an area of psychology that has barely been explored.

It seems reasonable to expect that additional anandamide-like compounds will also be discovered/made in the future, with potential implications for pathology and the treatment of numerous diseases.

CBD has been reported to exert beneficial effects in multiple disease models, as discussed in this review, and the recent FDA approval of CBD in rare forms of childhood epilepsy opens a door for further exploratory clinical investigations of the potential benefits/risks of this compound in other diseases affecting humans. If CBD is proven to be effective in other forms of epilepsy, or in various inflammatory, auto-immune, and neurological and psychiatric diseases, additional CBD analogs may be developed. Most forms of CBD are still illegal in the United States. Furthermore, the therapeutic dose of CBD showing efficacy in epileptic children is 20 mg/kg/day, which is consistent with benefits seen in preclinical studies with a 5–10-mg/kg daily dose in most disease models. It is, therefore, very important to emphasize that the large number of claims made with various CBD oils/products of unknown origin and uncontrolled composition containing very small doses of CBD (100–200 mg/bottle to be consumed for 4 weeks; equivalent to 0.005 mg/kg/day or less) are not supported by any preclinical or clinical studies. These unregulated CBD formulations may also contain harmful pesticides and heavy metals, and this poses a safety risk to their use.

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