

CANNABINOID RECEPTORS AND THEIR ENDOGENOUS AGONISTS

Christian C. Felder

Neuroscience Discovery, Eli Lilly Research Laboratories, Indianapolis, Indiana 46285;
e-mail: felder@lilly.com

Michelle Glass

Laboratory of Cellular and Molecular Regulation, National Institute of Mental Health,
Bethesda, Maryland 20892

KEY WORDS: marijuana, anandamide, fatty acid ethanolamide, cannabinoids, receptors

ABSTRACT

Marijuana has been in use for over 4000 years as a therapeutic and as a recreational drug. Within the past decade, two cannabinoid receptor types have been identified, their signal transduction characterized, and an endogenous lipid agonist isolated from mammalian tissues. The CB1 cannabinoid receptor is widely distributed in mammalian tissues, with the highest concentrations found in brain neurons. CB1 receptors are coupled to modulation of adenylate cyclase and ion channels. The CB2 receptor is found in cells of the immune system and is coupled to inhibition of adenylate cyclase. Both receptor types selectively bind Δ^9 -THC, the active principle in marijuana, and anandamide (arachidonylethanolamide), an endogenous cannabimimetic eicosanoid. Progress is being made in the development of novel agonists and antagonists with receptor subtype selectivity, mice with genetic deletion of the cannabinoid receptors, and receptor-specific antibodies, which should help in providing a better understanding of the physiological role of the cannabinoid receptors.

INTRODUCTION

For many centuries, marijuana has been used both recreationally, as a result of its psychoactivity, and medicinally. The drug's considerable therapeutic potential was documented as early as the fourth century BC (1, 2). During the rule of Emperor Chen Nung, the Chinese used marijuana for the treatment of malaria, constipation, rheumatic pains, absentmindedness, and female disorders. Use of

marijuana spread west from early Chinese culture to India and finally to Eastern Europe, where anecdotal information about its health benefits were finally put to scientific scrutiny early in the nineteenth century. Marijuana has been suggested as a treatment for a number of medical ailments, including nausea associated with chemotherapy, pain, migraine, epilepsy, glaucoma, hypertension, and the discomforts of child birth (see 3 for a review). More recently, its use as an appetite stimulant has been indicated in patients with cachexia or wasting disease observed, for example, in AIDS victims (4, 5). The recent Food and Drug Administration (FDA) approval of Marinol[®], an oral preparation of synthetic Δ^9 -tetrahydrocannabinol (Δ^9 -THC) (Figure 1), and the recent passage of propositions in California and Arizona allowing the medicinal use of smoked marijuana have renewed interest in the therapeutic potential of cannabinoids. The modern development of cannabinoids as therapeutic agents has been hampered largely because of their abuse potential and difficulties in separating the psychotropic effects from possible therapeutic effects.

CANNABINOID RECEPTORS

In the 1980s, scientific research on marijuana became significantly more molecular, attracting the attention of more biochemists and pharmacologists. Early evidence that a specific cannabinoid receptor was mediating the effects of Δ^9 -THC included studies demonstrating the ability of cannabinoids to inhibit signal transduction mediated through adenylate cyclase and cAMP formation (6, 7) and pharmacological studies showing enantiomeric specificity and structure-activity relationships for cannabinoid agonists (8). However, as a result of the highly lipophilic nature of cannabinoid compounds, traditional receptor binding techniques were problematic. The discovery of authentic cannabinoid binding sites began with the synthesis of the novel cannabinoid ligands that were significantly less lipophilic and more potent than Δ^9 -THC (9). Using potent cannabinoid agonists, such as CP-55,940 (Figure 1), it was shown that cannabinoids could inhibit adenylate cyclase in neuroblastoma cells (10, 11). This inhibition was GTP dependent and could be blocked with pertussis toxin, a finding consistent with a Gi-protein-coupled receptor. Radioligand binding studies using [³H]CP-55,940 subsequently confirmed the presence of cannabinoid binding sites in the brain (12, 13). The CB1 receptor was subsequently cloned from rat (14), and later from human (15) and mouse (16, 17). There appear to be some species differences in the chromosomal localization of the CB1 receptor gene: The gene for the bovine CB1 receptor has been localized to chromosome 9 (18), whereas the murine gene is reported to be found on chromosome 4 (19). Additionally a splice variant of the CB1 receptor, CB1a (20), has been described but appears to be a relatively minor component of the

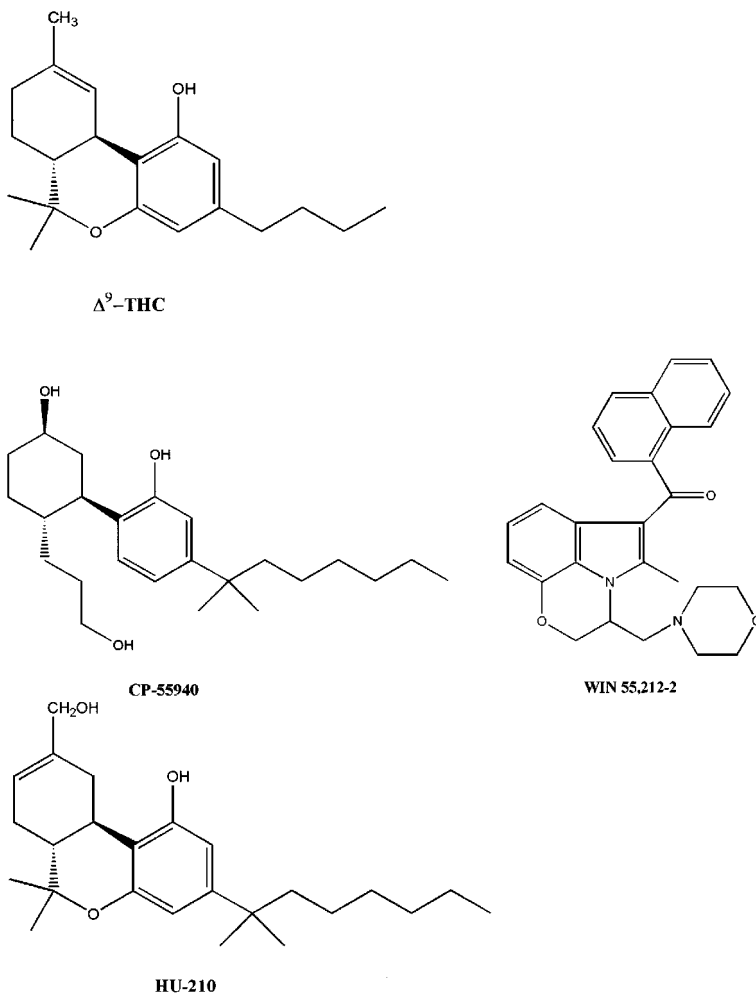


Figure 1 Agonists.

total CB1 receptor message. A second receptor subtype (CB2) exhibiting a low overall homology with the CB1 receptor (44%, with 68% in the helical regions) was cloned from human (21) and mouse (22). Both receptors have amino acids characteristic of G-protein-coupled receptors whose single polypeptide structure spans the plasma membrane seven times in a serpentine-like topology. No additional receptor subtypes have been identified to date, yet mice bearing genetic deletion of both CB1 and CB2 receptors have recently been bred, which

may provide information about additional members of this receptor family (24; N Buckley, T Bonner, A Zimmer & M Brownstein, personal communication).

Initially it was believed that the CB1 receptor was localized exclusively in the brain and testis, whereas the CB2 receptor was localized peripherally; however, it is gradually emerging that the distinction is not so simplistic. CB1 receptor distribution has been very well characterized in rat (25–27) and human brain (28, 29). The CB1 receptor exhibits a widespread distribution in the brain that correlates well with the known effects of cannabinoids on memory, perception, and the control of movement. It is expressed in high abundance in the hippocampus, associational cortical regions, the cerebellum, and the basal ganglia. Binding is sparse or absent from areas of brain stem, medulla, and thalamus, which might help explain the general lack of serious acute effects associated with marijuana abuse. In addition to the testis, the CB1 receptor has recently been suggested to be present peripherally in guinea pig small intestine (30), the mouse urinary bladder (31) and vas deferens (32), cerebral vascular smooth muscle cells (33), and pre-synaptically on sympathetic nerve terminals (34). CB1 receptor mRNA has been described in the adrenal gland, heart, lung, prostate, bone marrow, thymus, and tonsils (35, 36), although other research groups have reported the absence of CB1 mRNA in some of these peripheral regions (37). CB2 receptors are found in the marginal zone of the spleen (21, 37), in tonsils and in immune cells (B-cells, monocytes, T-cells, etc) (21, 35, 37) and possibly in primary cultures of rat microglia (38).

Pharmacology

The mechanism of action of marijuana remained unclear until chemical analyses of plant extracts revealed a principal active ingredient. First isolated as a “toxic red oil” late in the nineteenth century, the chemical structure of Δ^9 -THC, the principal psychoactive component in marijuana, was not fully established until 1964 (39). Availability of Δ^9 -THC in its pure form and the development of structural analogues led to controlled studies characterizing the pharmacological profile of the “high” experienced by human subjects as well as behavioral correlates observed in animals (8, 40). Several less potent but potentially important metabolites and related compounds are also found in marijuana, but much less is known about their therapeutic potential or their interactions with Δ^9 -THC.

A series of highly selective and potent cannabinoid receptor agonists were synthesized based on the three-attachment-site model of the benzofuran structure of Δ^9 -THC (41) or on the novel aminoalkylindol series of compounds (42) (see Table 1). [3 H]CP-55,940 was the first radioligand available and continues to be highly utilized. Only recently has the first cannabinoid receptor antagonist, SR141716A (Figure 2), been synthesized and shown to be selective for

Table 1 Summary of cannabinoid receptor signal transduction and pharmacology

	CB1	K_i (nM) ^a	CB2	K_i (nM)
Signal transduction	↓ cAMP ↓ Ca ²⁺ (N-, Q-type) ↑ K ⁺		↓ cAMP	
Endogenous agonists	AEA 2-AG	400 ± 120 ^b 472 ± 55 ^c	AEA 2-AG	1760 ± 360 ^b 1400 ± 172 ^c
Agonists	HU210 CP55,940 Δ ⁹ -THC WIN55212-2	0.06 ± 0.01 ^b 3.72 ± 0.01 ^b 53 ± 8 ^b 62 ± 31 ^b	HU210 CP55,940 WIN55212-2 Δ ⁹ -THC	0.5 ± 0.04 ^b 2.55 ± 0.28 ^b 3.3 ± 0.4 ^b 75 ± 8 ^c
Antagonists	SR141716A	12 ± 2 ^b	SR144528	0.60 ± 0.13 ^d

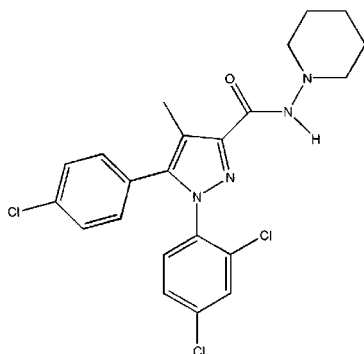
^aAgonists are listed in order of descending affinity. The K_i values were derived against [³H]-CP55,940 in a variety of models; ^bstably transfected L-CB1 or CHO-CB2 cell lines (52); ^ctransiently transfected COS-7 cells (100); ^dstably transfected CHO-CB2 cells (J Barth, personal communication). AEA: anandamide, 2-AG: 2-arachidonyl glycerol.

the CB1 receptor (43). This was followed by promising leads for CB2 receptor selective agonists, such as the 1-deoxy analogue of HU-210 (11-hydroxy-Δ⁸-THC-dimethyl heptyl) (Figure 1) (44) and two indole analogues (45). A CB2 receptor selective antagonist, SR144528 (Figure 2), has just been reported (46). Additional cannabinoid selective compounds will be necessary to more fully understand this family of receptors and to probe for other possible subtypes.

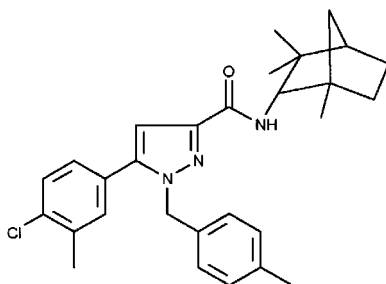
Signal Transduction

As might be expected with their low sequence homology, CB1 and CB2 receptors vary considerably in their coupling to signal transduction pathways. Both the CB1 and CB2 receptors inhibit adenylate cyclase via a pertussis toxin-sensitive G protein. Recently it has been shown that CB1 but not CB2 receptors couple to the stimulation of adenylate cyclase under certain conditions (47). Furthermore, CB1 but not CB2 receptors have been shown to inhibit N- and Q-type calcium channels (48–52) and to activate inwardly rectifying potassium channels (48, 52). Inhibition of N-type calcium channels decreases neurotransmitter release from several tissues. It may therefore be the mechanism by which cannabinoids inhibit acetylcholine release in the hippocampus (53, 54); noradrenaline release at the sympathetic nerve terminals (34) and centrally in the hippocampus, cortex, and cerebellum (55); and glutamate release in cultured hippocampal neurons (56).

Many of the intracellular effects of cannabinoids can be explained by their ability to activate G_i proteins or inhibit cAMP. Inhibition of calcium channels by CB1 receptors is pertussis toxin-sensitive, but independent of cAMP



SR 141716A



SR144528

Figure 2 Antagonists.

inhibition suggestive of a direct G-protein mechanism. Stimulation of potassium channels is pertussis toxin-sensitive and thought to be mediated by inhibition of cAMP (57). Attenuation of inducible nitric oxide synthase gene expression and nitric oxide production by cannabinoids occurs at least in part through the inhibition of cAMP signaling and may in turn lead to cannabinoid receptor-mediated inhibition of immune function, which is thought to be a CB2 receptor-mediated process (58, 59). Furthermore, cannabinoid agonists activate mitogen-activated protein (MAP) kinases via a G protein but not via cAMP-dependent mechanisms (60). MAP-kinase activation may be an intermediate step in the cannabinoid receptor-mediated induction of the transcription factor Krox 24 (60–62).

Cannabinoid receptor-independent effects of cannabinoid agonists have also been observed. At concentrations well above the agonist dissociation constants

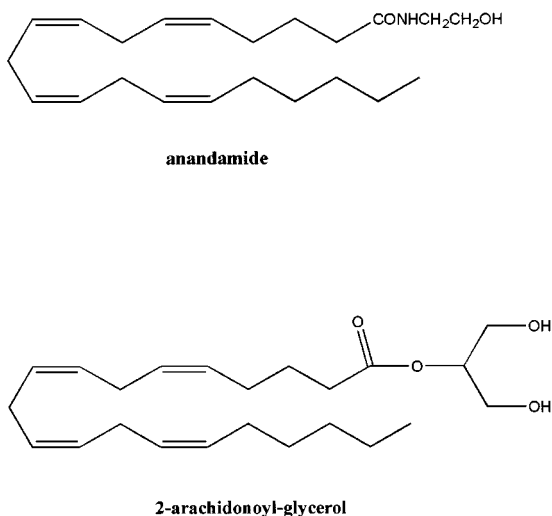


Figure 3 Endogenous agonists.

($K_d > 1 \mu\text{M}$), cannabinoid agonists stimulate the release of arachidonic acid, the inhibition of arachidonic acid re-acylation, and release of cytoplasmic calcium stores (63–66). The mechanism of the cannabinoid agonist-mediated production of arachidonic acid and eicosanoids has been somewhat controversial. Recently it was shown that anandamide (Figure 3) and Δ^9 -THC stimulated arachidonic acid release in cortical astrocytes and that this could be blocked by the CB1 receptor antagonist SR141716A (67). However, the concentrations of agonists and antagonists required in this study were well above their affinity constants for the CB1 receptor, and cannabinoid expression in astrocytes has not been clearly demonstrated. Antisense oligonucleotide probes for the CB1 and CB2 receptors were shown to block arachidonic acid release, thereby providing additional evidence that in some cells, cannabinoid receptors may mediate eicosanoid production (68). It is unclear whether these effects are of physiological significance in the response to cannabinoids in vivo.

ENDOGENOUS CANNABIMIMETIC EICOSANOIDS

The discovery of specific cannabinoid receptors for Δ^9 -THC suggested that an endogenous agonist that stimulates the CB1 receptor may also be present in the brain. Reasoning that an endogenous agonist may have hydrophobic properties similar to those of cannabinoid agonists, Devane, Mechoulam, and coworkers (69) focused their search in organic solvent extracts of porcine brain. Their

hypothesis proved correct when they discovered a lipid molecule, anandamide, that displaced specific binding of a radiolabeled cannabinoid agonist in rat brain membranes and functionally inhibited electrically induced twitch response in murine vas deferens (69). The structure was shown to be an eicosanoid consisting of the 20 carbon fatty acid, arachidonic acid, coupled to ethanolamine through an amide linkage. The name of anandamide is based on the Indian Sanskrit word *ananda*, which is defined as “bringer of inner bliss and tranquility.” Interestingly, arachidonyl ethanolamide was discovered independently by Johnson and coworkers during a search for ligands for 1,4-dihydropyridine binding sites on L-type voltage-dependent calcium channels (70).

Further evidence for anandamide's role as an endogenous cannabimimetic agonist included data showing binding and functional stimulation of the CB1 receptor (71–73) and CB2 receptor (22, 52, 74). In these studies, anandamide exhibited almost identical actions, both receptor and nonreceptor mediated, as classical cannabinoid agonists including displacement of cannabinoid agonist binding, inhibition of adenylate cyclase activation, inhibition of N-type Ca^{2+} channels, and stimulation of cannabinoid receptor-independent mobilization of arachidonic acid and calcium. Though some variation in binding affinity has been observed between laboratories, anandamide displays a midnanomolar affinity for the CB1 receptor in both CB1 receptor-expressing cell lines and rat brain homogenates (72, 73, 75–77) (Table 1). Behavioral effects seen with Δ^9 -THC—such as hypothermia, analgesia, hypomobility, and catalepsy—were mimicked with anandamide (78–80), further supporting its role as an endogenous cannabinoid agonist. However, relatively high concentrations of anandamide were required to exert the behavioral effects possibly due to metabolism, absorption into adipose, or rapid uptake. The relatively low affinity of anandamide for the CB1 receptor raises the possibility that other more potent agonists exist for the CB1 receptor in the brain or, conversely, that higher-affinity binding sites than the CB1 receptor exist. Indeed, cannabinoid agonist-independent effects of anandamide have been observed, such as inhibition of gap junction (81), peripheral pressor effects on the cardiovascular system (82), and a possible role as an endothelium-derived vasorelaxation factor (83).

Anandamide Pharmacology

Initial observations of the structural requirements for anandamide-like lipids as potential agonists for the CB1 receptor revealed a strict requirement for ethanolamine coupled to fatty acids of 20 to 22 carbon lengths (71). Other than anandamide, three additional fatty acid ethanolamides were proposed as potential cannabinoid receptor agonists [ethanolamides of dihomo- γ -linolenic acid (C20:3 n-6), adrenic acid (C22:4 n-6), and mead acid (C20:3 n-9)].

Subsequently, dihomono- γ -linolenyl ethanolamide and adrenylethanolamide were isolated from mammalian brain (84) and shown to stimulate cannabinoid-like behavioral effects (85). However, it is unclear if they play a role in normal physiology, since it is unknown if sufficient levels of these two endogenous agonists occur in the mammalian brain. Several additional congeners of anandamide have since been synthesized to provide an understanding of the structural requirements for fatty acylethanolamide binding to cannabinoid receptors. For example, congeners were synthesized to address specific questions such as the structural relationship of anandamide to the cannabinoid pharmacophore (86, 87) and to oxidized eicosanoids (75, 77), and the role of the ethanolamine head group (88). Additional congeners were synthesized in order to stabilize the lipid structure (89) or make it resistant to hydrolysis (90), thus preventing its loss of activity. Results of these studies support anandamide as an endogenous agonist for the CB1 receptor and emphasize that anandamide's structure is optimal as a cannabinoid receptor agonist compared to related structures tested to date. Anandamide has also been shown to act as an agonist at the CB2 receptor suggesting it may act as an endogenous agonist in the peripheral immune system (52, 74).

Anandamide Localization

Anandamide was first quantified in porcine whole brain [0.4 pmol/mg protein (69)] and subsequently identified in whole sheep and cow brain [approximately 1.7 and 1.0 pmol/mg protein, respectively (91)], rat testis [0.06 pmol/mg protein (92)], and widely distributed in the brain and periphery of rat and humans (93). Organic solvent extracts of rat and postmortem human brain revealed anandamide in all brain areas tested with highest levels found in the hippocampus, striatum, cerebellum, and cortex—areas of high-density CB1 receptor binding. However, some mismatch was found between anandamide levels and CB1 receptor distribution. For example, anandamide was found in the thalamus where CB1 receptors are in very low abundance (25), suggesting that additional roles for anandamide may exist in the brain. Anandamide was also found in the spleen, suggesting that it may be an endogenous agonist for CB2 receptors, although levels were below those measured in the brain (93). The concentration of anandamide in the plasma was determined to be 4.4 pmol/ml, or 4.4 nM, which is similar to levels of dopamine found in rat plasma (94). The presence of anandamide in plasma suggests that anandamide may be synthesized in a particular organ or tissue and released to the circulation to act on distant cells.

Anandamide was not the first fatty acid ethanolamide to be discovered. Palmitoylethanolamide was first isolated in 1957 from soybean lethicin and tested as an antiinflammatory agent (95) and its synthesis later observed in rat brain (96). Additional fatty acid ethanolamides that increase during ischemia

were later identified in studies of the metabolism of *N*-acylphospholipids in canine myocardium and brain (97). However, arachidonylethanolamide was never found in these preparations. It was first proposed that release of anandamide and related fatty acid ethanolamides might be regulated by neurotransmitters (98) following the observation that glutamate agonists and calcium ionophore increased levels of anandamide-related fractions from neurons in culture. A variety of fatty acid ethanolamides with carbon chain lengths of 14–22 have also been identified in rat testes, and brain tissue from sheep, cow, rat, and pigs (91, 92, 99). However, none of the fatty acid ethanolamides, except for anandamide, display any significant affinity for the cannabinoid receptors. It remains to be determined if these molecules have their own biological activity and binding sites.

The discovery of an endogenous lipid molecule acting as an agonist at a G-protein-coupled receptor that modulates brain function stimulated several questions that are currently being considered by a number of laboratories. What other possible related molecules exist in brain or peripheral tissue that can act on the cannabinoid receptors? If these molecules exist, do they each have their own receptors, or do they all bind to cannabinoid receptors with differing affinities? Considering the relatively low affinity of anandamide for the CB1 and CB2 receptors, do higher-affinity endogenous ligands exist, and are they structurally similar to anandamide? Do fatty acid ethanolamides represent a novel class of lipid neurotransmitter, since anandamide is too hydrophobic to be stored in synaptic vesicles? What is the mechanism of anandamide storage and release, and is it a synaptic event regulated by other neurotransmitters?

Although elements of these and other questions are currently under investigation, considerable effort has been focused on the possibility of additional endogenous cannabinoid agonists. A promising candidate for an endogenous cannabinoid receptor agonist to emerge from this research is the 2-arachidonylglycerol (2-AG) (Figure 3) first isolated from canine gut (100) and later observed in mouse neuroblastoma cells (101, 102). However, its affinity for both the CB1 and CB2 receptor is at least as low as that of anandamide (103) (see Table 1), and its distribution not well characterized. More recently, 2-AG has been found to be released from brain neurons following stimulation with calcium ionophore, possibly through activation of phosphatidylinositol-specific phospholipase C (104). In these neurons, 2-AG inhibited forskolin-stimulated cAMP accumulation through the CB1 receptor, but displayed a relatively low affinity ($K_i = 2 \mu\text{M}$). However, levels of 2-AG were found to be approximately 200 times higher than anandamide in brain samples (104). The proposed synthesis pathway for the generation of 2-AG is shown in Figure 4.

Classical neurotransmitters are defined as small molecules that are stored in synaptic vesicles that are released to the synaptic cleft following an appropriate

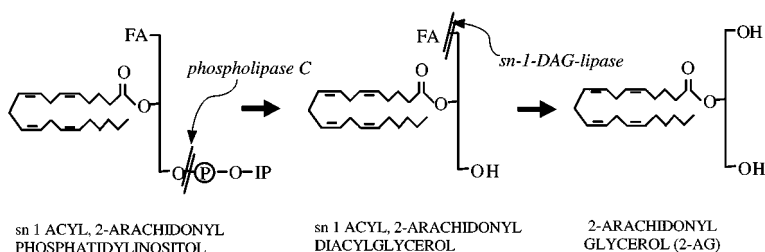


Figure 4 Proposed pathway for the biosynthesis of sn 2 arachidonylglycerol (2-AG). Neuronal activity leads to a rise in intracellular calcium that triggers the activation of phospholipase C, converting sn 2-arachidonylphosphatidylinositol, to a diacylglycerol by cleavage of the phosphodiester bond. The sn 1-acyl group is then removed by DAG-lipase (presumably specific for the sn 1 position), producing 2-AG. It has been proposed that a mono-acylglycerol lipase inactivates this compound, by removal of the arachidonyl group. (See References 101 and 104 for further details.)

signal, usually a membrane depolarizing event. In addition, neurotransmitters bind to a receptor protein, elicit functional responses such as generation of second messengers, and finally, are removed from the synapse through uptake or metabolism. Anandamide deviates from the classical definition of a neurotransmitter as a result of its hydrophobicity. As a lipophilic compound, anandamide would not be stored within the synaptic vesicle cytoplasm, but would diffuse freely across membranes. Therefore, anandamide might reside in the membrane in phospholipid precursor form to be released following activation of an appropriate phospholipase or related enzyme.

Biosynthesis and Metabolism

Two hypotheses have been explored for anandamide biosynthesis. The first suggests that the release of both arachidonic acid and ethanolamine might occur following activation of phospholipase A₂ and phospholipase D, respectively (105, 106). A putative anandamide synthase would then create the amide bond to form anandamide. However, kinetic measurements suggest that concentrations of substrates higher than may be achieved physiologically would be necessary for this reaction to proceed (105, 106). An alternative pathway based on the early studies of Schmidt and coworkers (97) has been proposed in rat testes (92) and brain (99, 107) (Figure 5). The formation of *N*-arachidonylphosphatidylethanolamine occurs through an acyl transferase that would move arachidonic acid from the first or possibly second position of a donor phospholipid to form an amide bond at the ethanolamine head group in the third position of phosphatidylethanolamine. Release of anandamide would then occur following activation of phospholipase D. The second hypothesis for anandamide formation appears to have the most support to date. Possible

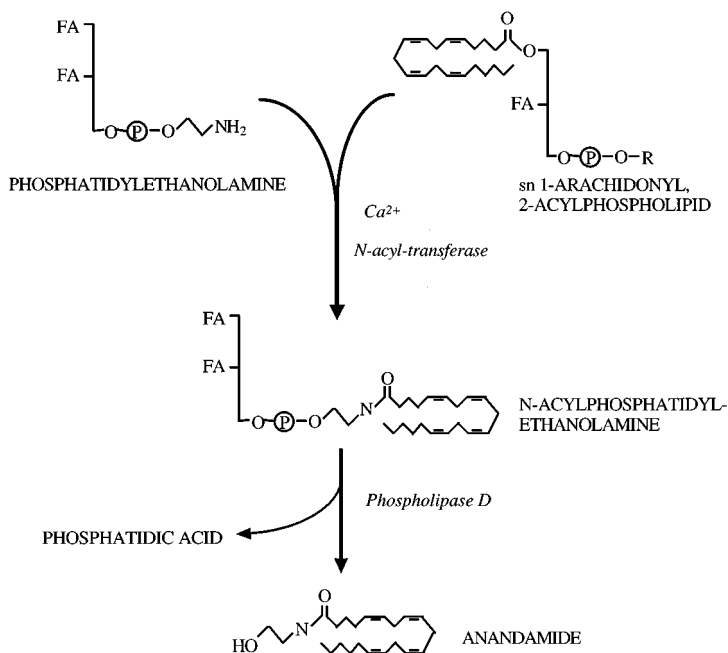


Figure 5 Proposed pathway for the biosynthesis of anandamide. Rises in intracellular calcium trigger the transfer of arachidonic acid from the *sn* 1 position of an *N*-acylphospholipid, to the amine group of phosphatidylethanolamine. Phospholipase D then cleaves the distal phosphodiester bond to release anandamide.

interactions between the synthesis pathways for anandamide and 2-AG are shown in Figure 6.

As a putative neurotransmitter, the removal of anandamide may occur through either uptake or metabolism. Selective and saturable anandamide uptake by a sodium- and energy-independent mechanism has been observed in cortical neurons in primary culture (98). Little progress has been made on identification of this transporter. Specific inhibitors of this process may help elucidate the physiological role of anandamide (108). Enzymatic degradation of anandamide has been observed by an amidohydrolase that also degrades a sleep-inducing oleioylamide first isolated from sleep-deprived cats. This enzyme has been isolated and cloned from rat liver (109). Amidohydrolase activity has been found in the intracellular compartments of neurons and therefore diffusion or uptake of anandamide into the cytoplasm is a prerequisite for degradation. Recently, inhibitors of the amidase enzyme have been developed (110–112).

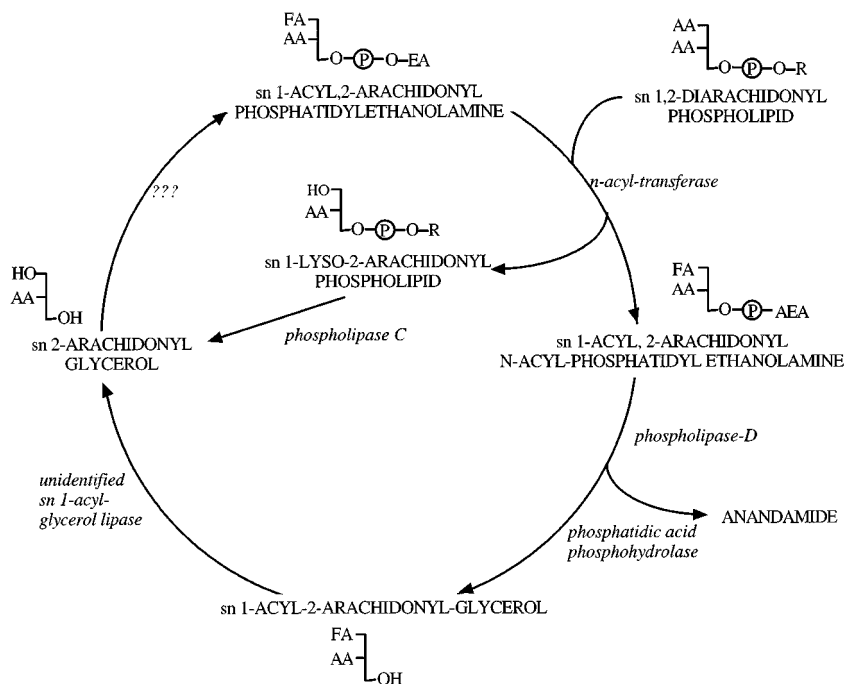


Figure 6 Possible interactions between the synthesis pathways of anandamide and 2-arachidonylglycerol (2-AG). If the initial phosphatidylethanolamine has arachidonic acid in the *sn* 2 position, both anandamide and 2-AG can be synthesized in the same pathway. Following the removal of anandamide from the *N*-acyl-phosphatidylethanolamine, the remaining phosphodiester bond on the phospholipid backbone can be cleaved to produce diacylglycerol with arachidonic acid in the *sn* 2 position. This can then be converted to 2-AG by the pathway described in Figure 1. Alternatively, if the arachidonic acid donor for the synthesis of anandamide is a diarachidonylphospholipid, then transfer of arachidonic acid from the *sn* 1 position of the donor would result in a *sn* 1-lyso-2 arachidonylphospholipid. Cleavage of the phosphodiester bond would then produce diacylglycerol, which could be converted to 2-AG. Salvage and resynthesis pathways have not been described but are proposed.

CLINICAL RELEVANCE

Cannabinoids as Potential Therapeutic Agents

Recent passage of Arizona and California state government legislation allowing physician-prescribed use of marijuana has refocused attention on the potential therapeutic uses of this drug. Many studies were conducted in the past three decades investigating the clinical effectiveness of both smoked marijuana and oral Δ^9 -THC, in multiple sclerosis, spasticity, nausea for cancer chemotherapy,

and glaucoma (see 113 for a review). Although many patients complained of toxicity (usually in the form of psychoactivity) regardless of the delivery form, Δ^9 -THC was found to be useful in both cancer chemotherapy and as an appetite stimulant. Smoked marijuana has many inherent problems in clinical trials, particularly in achieving accurate control of dosage. In addition, smoking marijuana exposes patients to 50% higher levels of procarcinogen benz- α -pyrene than does smoking tobacco (114), and to carboxyhemoglobin levels and tar levels that are five times higher and three times higher than those produced by tobacco smoking, respectively (115). These problems can largely be overcome by the use of an oral preparation. Oral Δ^9 -THC (Marinol or Dronabinol) received FDA approval in 1984 for nausea from cancer chemotherapy, and in 1992 for AIDS patients to combat weight loss. Low doses of oral Δ^9 -THC have been demonstrated to be sufficient to produce appetite stimulation or antiemesis in the absence of significant psychotropic effects (116–119). However, many patients prefer smoking Δ^9 -THC to an oral preparation as it allows them better control of the dosage and has a more rapid onset.

Recent understanding of the pharmacology and molecular biochemistry of cannabinoid receptors should help refocus both clinical and basic research efforts. High-potency cannabinoid analogues may circumvent the problems caused by smoked marijuana, such as the difficulties in controlling dosing and the inherent hazards of smoking. Furthermore, receptor-selective agonists may eliminate many of the adverse effects of Δ^9 -THC by targeting only one type of receptor (for example, immunosuppression may be avoided by using a selective CB1 agonist, whereas CB2 agonists should not produce sedative or psychotropic effects). Much of the political and public objection to the use of Δ^9 -THC or marijuana as a therapy centers around its abuse potential and the belief by some that it serves as a “gateway” drug leading users to “harder” drugs of abuse. Many therapeutic drugs have abuse potential, yet this does not invalidate their role in current therapies. While there is some preliminary evidence for cannabinoids activating the reward pathways in the brain (120), most investigators have failed to find addictive or reinforcing effects of cannabinoids in animal models. Unlike cocaine or heroin, cannabinoid agonists produce conditioned place aversion even at low doses (121, 122) and anxiogenic effects (123). Furthermore, animals will not self-administer cannabinoids (124–126), and a lack of cross sensitization between cocaine (127) or amphetamine (128) and cannabinoids has also been demonstrated.

Cannabinoid Receptor Alterations in Disease States

The isolation of anandamide and the cannabinoid receptors raise new questions as to the role of cannabinoids in the etiology of a range of disorders. There have been few studies to date linking cannabinoid receptors causally to any disorders, and these have been somewhat controversial. Recently, available

antagonists have allowed investigators to begin examining the physiological role of the endogenous cannabinoid system by blocking receptor function with the application of an antagonist. Some studies have suggested that the CB1 receptor antagonist, SR141716A, can produce place preference (129), suggesting that endogenous cannabinoids serve normally to suppress reward or to induce aversion. However, others have observed increased anxiety in response to SR141716A administration (130). Injection of SR141716A in rats has been demonstrated to increase motor activity at high doses (131). Agonists and antagonists to the CB2 receptor have only very recently been developed and have not been studied in any detail.

The behavioral responses to cannabinoid agonists and antagonists, and the high level of cannabinoid receptors in the movement centers of the brain, such as the cerebellum and the basal ganglia, may indicate a role for cannabinoids in the control of movement and possibly movement disorders. Cannabinoid receptor binding has been demonstrated to decrease very early in the pathology of Huntington's disease (132, 133). Cannabinoids may be useful in the treatment of the hyperkinetic aspects of Huntington's disease given their ability to produce hypomobility in animals. However, the loss of the receptor so early on in the disease process may preclude the cannabinoids from being effective. Studies are under way to investigate the levels of anandamide in early Huntington's disease brains to determine if this could be contributing to the receptor alterations. No correlation has been found between CB1 receptor levels and any other neurological disorders to date (29). Perhaps as more is understood about the synthesis, release, and inactivation of anandamide, it may become possible to manipulate the endogenous levels of anandamide. Such a strategy has proven therapeutically useful with other neurotransmitters, such as in the case of selective serotonin reuptake inhibitors. It may be possible to alter anandamide levels to a concentration that avoids psychoactive side effects. Another alternative is the use of low-dose cannabinoids as an adjunct therapy. For example, subtherapeutic doses of cannabinoids have been demonstrated to synergize with opioids in producing antinociception (134).

CONCLUSION

It is now thought that most of the effects of marijuana are mediated through the interaction of Δ^9 -THC with cannabinoid receptors. However, a clear understanding of cannabinoid receptor physiology has been elusive. Recent advances in the understanding of the molecular pharmacology and biochemistry of cannabinoid receptors and their lipid endocannabinoids should provide a better approach for further basic research and possibly clinical trials leading to a more effective therapeutic. The sizable number of cannabinoid agonists developed over the past three decades can now be applied to cannabinoid research

in conjunction with recently developed antagonists and knockout mice lacking cannabinoid receptors, thereby providing a better understanding of this widely distributed and abundant receptor family. It is curious that marijuana, which has one of the longest therapeutic histories and continues to be broadly utilized, is one of the most poorly understood therapeutics.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Miles Herkenham, Dave Leander, and Julius Axelrod for critical reading of the manuscript, and Eileen Briley for assistance in preparation of the manuscript.

Visit the *Annual Reviews* home page at
<http://www.AnnualReviews.org>.

Literature Cited

1. Mechoulam R. 1986. The pharmacology of cannabis sativa. In *Cannabinoids as Therapeutic Agents*, ed. R. Mechoulam, pp. 1–19. Boca Raton, FL: CRC
2. Zias J, Stark H, Sellgman J, Levy R, Werker E, et al. 1993. Early medical use of cannabis. *Nature* 363:215
3. Grinspoon L, Bakala JB. 1993. *Marijuana. The Forbidden Medicine*. New Haven, CT: Yale Univ. Press
4. Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, et al. 1995. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *Pain Symptom Manage.* 10:89–97
5. Grinspoon L, Bakalar JB, Doblin R. 1995. Marijuana, the AIDS wasting syndrome, and the U.S. government [letter]. *N. Engl. J. Med.* 333:670–71
6. Howlett AC. 1984. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nandriadiol compounds. *Life Sci.* 35:1803–10
7. Howlett AC, Fleming RM. 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response of neuroblastoma cell membranes. *Mol. Pharmacol.* 26:532–38
8. Dewey WL. 1986. Cannabinoid pharmacology. *Pharmacol. Rev.* 38:151–78
9. Johnson MR, Melvin LS. 1986. The discovery of non-classical cannabinoid analgesic. In *Cannabinoids as Therapeutic Agents*, ed. CR Mechoulam, pp. 121–45. Boca Raton: CRC
10. Howlett AC, Johnson MR, Melvin LS, Milne GM. 1988. Nonclassical cannabinoid analgesics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol. Pharmacol.* 33:297–302
11. Howlett AC, Qualy JM, Khachatrain LL. 1986. Involvement of Gi in the inhibition of adenylate cyclase by cannabinoid drugs. *Mol. Pharmacol.* 29:307–13
12. Harris LS, Carchman RA, Martin BR. 1978. Evidence for the existence of specific cannabinoid binding sites. *Life Sci.* 22:1131–37
13. Devane WA, Dysarc FA III, Johnson MR, Mevin LS, Howlett AC. 1988. Determination and characterisation of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* 34:605–13
14. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. 1990. Structure of cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346:561–64
15. Gerard CM, Mollereau C, Vassart G, Parmentier M. 1991. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* 279:129–34
16. Abood ME, Ditto KE, Noel MA, Showalter VM, Tao Q. 1997. Isolation and expression of a mouse CB1 cannabinoid receptor gene—comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma-cells. *Biochem. Pharmacol.* 53:207–14
17. Chakrabarti A, Onaivi ES, Chaudhuri G. 1995. Cloning and sequencing of a cDNA encoding the mouse brain type

- cannabinoid receptor protein. *DNA Sequence* 5:385–88
18. Pfister-Genskow M, Weesner GD, Hayes H, Eggen A, Bishop MD. 1997. Physical and genetic localization of the bovine cannabinoid receptor (CNR1) gene to bovine chromosome 9. *Mamm. Genome* 8:301–2
 19. Stubbs L, Chittenden L, Chakrabarti A, Onaivi E. 1996. The gene encoding the central cannabinoid receptor is located in proximal mouse chromosome-4. *Mamm. Genome* 7:165–66
 20. Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, et al. 1995. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J. Biol. Chem.* 270:3726–31
 21. Munro S, Thomas KL, Abu-Shaar M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365:61–65
 22. Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, et al. 1996. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochem. Biophys. Acta* 1307:132–36
 23. Deleted in proof
 24. Buckley NE, Mezey E, Bonner T, Felder C, Glass M, et al. 1997. *Development of a CB2 knockout mouse. Symposium on Cannabinoids*, 1:57. Burlington, VT: Int. Cannabinoid Res. Soc.
 25. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, et al. 1991. Characterization and localization of cannabinoid receptors in the rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* 11:563–83
 26. Herkenham M, Lynn AB, de Costa BR, Richfield EK. 1991. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* 547:267–74
 27. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, et al. 1990. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA* 87:1932–36
 28. Glass M, Faull RLM, Dragunow M. 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study on the fetal, neonatal and adult human brain. *Neuroscience* 77:299–318
 29. Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M. 1994. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* 63:637–52
 30. Pertwee RG, Fernando SR, Nash JE, Coutts AA. 1996. Further evidence for the presence of cannabinoid CB1 receptors in guinea pig small intestine. *Br. J. Pharmacol.* 118:2199–205
 31. Pertwee RG, Fernando SR. 1996. Evidence for the presence of cannabinoid receptors in mouse urinary bladder. *Br. J. Pharmacol.* 118:2056–58
 32. Pertwee RG, Joedigwe G, Hawksworth GM. 1996. Further evidence for the presence of cannabinoid CB1 receptors in mouse vas-deferens. *Eur. J. Pharmacol.* 296:169–72
 33. Hillard CJ, Gebremedhin D, Lange AR, Campbell WB, Harder DR, et al. 1997. *Cannabinoid receptors (CB1) of cerebral arterial muscle cells inhibit L-type calcium current: Does the CB1 receptor play a role in the regulation of cerebral blood flow. Symposium on Cannabinoids*, 1:73. Burlington, VT: Int. Cannabinoid Res. Soc.
 34. Ishac EJN, Jiang L, Lake KD, Varga K, Abood ME, et al. 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* 118:2023–28
 35. Galiegue S, Mary S, Marchand J, Dusossoy D, Carriere D, et al. 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232:54–61
 36. Rice W, Shannon JM, Burton F, Fieldelley D. 1997. Expression of a brain-type cannabinoid receptor (CB1) in alveolar type-II cells in the lung—regulation by hydrocortisone. *Eur. J. Pharmacol.* 327:227–32
 37. Schatz ARE, Lee M, Condie RB, Pulaski JT, Kaminski NE. 1997. Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* 142:278–87
 38. Kearns CS, Hillard CJ. 1997. *Rat microglial cells express the peripheral-type cannabinoid receptor (CB2) which is negatively coupled to adenylyl cyclase. Symposium on Cannabinoids*, 1:61. Burlington, VT: Int. Cannabinoid Res. Soc.
 39. Gaoni Y, Mechoulam R. 1964. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86:1646–47

40. Hollister LE. 1986. Health aspects of cannabis. *Pharmacol. Rev.* 38:1–20
41. Martin BR, Compton DR, Prescott WR, Barrett RL, Razdan RK. 1995. Pharmacological evaluation of dimethylheptyl analogs of delta 9-THC: reassessment of the putative three-point cannabinoid-receptor interaction. *Drug Alcohol Depend.* 37:231–40
42. Eissenstat MA, Bell MR, D'Ambra TE, Alexander EJ, Daum SJ, et al. 1995. Aminoalkylindoles: structure-activity relationships of novel cannabinoid mimetics. *J. Med. Chem.* 38:3094–105
43. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, et al. 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 350:240–44
44. Huffman JW, Yu S, Showalter V, Abood ME, Wiley JL, et al. 1996. Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. *J. Med. Chem.* 39:3875–77
45. Gallant M, Dufresne C, Gareau Y, Guay D, Leblanc Y, et al. 1996. New class of potent ligands for the human peripheral cannabinoid receptor. *Biol. Med. Chem. Lett.* 6:2263–68
46. Barth F, Rinaldi-Carmona M, Millan J, Derocq J, Bouaboula M, et al. 1997. SR144528, a potent and selective antagonist of the CB2 receptor. *Symposium on Cannabinoids*, 1:11. Burlington, VT: Int. Cannabinoid Res. Soc.
47. Glass M, Felder CC. 1997. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs-linkage to the CB1 receptor. *J. Neurosci.* 17:5327–33
48. Mackie K, Lai Y, Westenbroek R, Mitchell R. 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in ATT20 cells transfected with rat brain cannabinoid receptors. *J. Neurosci.* 15:6552–61
49. Mackie K, Hille B. 1992. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. USA* 89:3825–29
50. Caulfield MP, Brown DA. 1992. Cannabinoid receptor agonists inhibit CA current in N9108-15 neuroblastoma cells via a pertussis toxin sensitive mechanism. *Br. J. Pharmacol.* 106:231–32
51. Pan XH, Ikeda SR, Lewis DL. 1996. Rat brain cannabinoid receptor modulates N-type Ca^{2+} channels in a neuronal expression system. *Mol. Pharmacol.* 49:707–14
52. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, et al. 1995. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.* 48:443–50
53. Gifford AN, Ashby CR. 1996. Electrically-evoked acetylcholine-release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN55212–2, and is potentiated by the cannabinoid antagonist, SR141716A. *J. Pharmacol. Exp. Ther.* 277:1431–36
54. Gessa GL, Mascia MS, Casu MA, Carta G. 1997. Inhibition of hippocampal acetylcholine release by cannabinoids—reversal by SR141716A. *Eur. J. Pharmacol.* 327:R1–R2
55. Schlicker E, Timm J, Görg T, Göthert M. 1997. Cannabinoid receptor-mediated inhibition of noradrenaline release and cAMP accumulation in the guinea-pig and human brain. *Symposium on Cannabinoids*, 1:63. Burlington, VT: Int. Cannabinoid Res. Soc.
56. Shen MX, Piser TM, Seybold VS, Thayer SA. 1996. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J. Neurosci.* 16:4322–34
57. Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S. 1995. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP dependent process. *J. Pharmacol. Exp. Ther.* 273:734–43
58. Jeon YJ, Yang KH, Pulaski JT, Kaminski NE. 1996. Attenuation of inducible nitric oxide synthase gene-expression by delta(9)-tetrahydrocannabinol is mediated through the inhibition of nuclear factor-kappa-B/Rel activation. *Mol. Pharmacol.* 50:334–41
59. Coffey RG, Yamamoto Y, Snella E, Pross S. 1996. Tetrahydrocannabinol inhibition of macrophage nitric-oxide production. *Biochem. Pharmacol.* 52:743–51
60. Bouaboula M, Poinotchazel C, Bourrie B, Canat X, Calandra B, et al. 1996. Activation of mitogen-activated protein-kinases by stimulation of the central cannabinoid receptor CB1. *Biochem. J.* 312:637–41
61. Bouaboula M, Bourrie B, Rinaldi-Carmona M, Shire D, Le Fur G, et al. 1995. Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. *J. Biol. Chem.* 270:13973–80
62. Glass M, Dragunow M. 1995. Selective

- induction of Krox 24 in striosomes by a cannabinoid agonist. *NeuroReport* 6:241–44
63. Reichman M, Nen W, Hokin LE. 1991. Delta-9-tetrahydrocannabinol inhibits arachidonic acid acylation of phospholipids and triacylglycerols in guinea pig cerebral cortex slices. *Mol. Pharmacol.* 40:547–55
 64. Reichman M, Nen W, Hokin LE. 1988. Delta-9-tetrahydrocannabinol increases arachidonate levels in guinea pig cerebral cortex slices. *Mol. Pharmacol.* 34:823–28
 65. Burnstein S, Hunter SA. 1978. Prostaglandins and cannabis. VI. Release of arachidonic acid from HeLa cells by delta-1-tetrahydrocannabinol and other cannabinoids. *Biochem. Pharmacol.* 27:1275–80
 66. Felder CC, Veluz JS, Williams HL, Briley EM, Matsuda LA. 1992. Cannabinoid agonists stimulate both receptor and non-receptor mediated signal transduction pathways in cells transfected and expressing cannabinoid receptor clones. *Mol. Pharmacol.* 42:838–45
 67. Shivachar AC, Martin BR, Ellis EF. 1996. Anandamide- and delta9-tetrahydrocannabinol-evoked arachidonic acid mobilization and blockade by SR141716A [N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride]. *Biochem. Pharmacol.* 51:669–76
 68. Hunter SA, Burnstein SH. 1997. Receptor mediation in cannabinoid stimulated arachidonic acid mobilization and anandamide synthesis. *Life Sci.* 60:1563–73
 69. Devane WA, Hanus L, Breuer A, Pertwee RG, Severson LA, et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258:1946–49
 70. Johnson DE, Heald SL, Dally RD, Janis RA. 1993. Isolation, identification and synthesis of an endogenous arachidonic amide that inhibits calcium channel antagonist 1,4-dihydropyridine binding. *Prostaglandins Leukot. Essent. Fatty Acids* 48:429–37
 71. Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, et al. 1993. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor mediated signal transduction. *Proc. Natl. Acad. Sci. USA* 90:7656–66
 72. Vogel Z, Barg J, Levy R, Saya D, Heldman E, et al. 1993. Anandamide, a brain endogenous compound interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *J. Neurochem.* 61:352–55
 73. Childers SR, Sexton T, Roy MB. 1994. Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem. Pharmacol.* 47:711–15
 74. Slipetz DM, O'Neill GP, Favreau L, Dufresne C, Gallant M, et al. 1995. Activation of the human peripheral cannabinoid receptor results in inhibition of adenylate cyclase. *Mol. Pharmacol.* 48:352–61
 75. Pinto JC, Potie F, Rice KC, Boring D, Johnson MR, et al. 1994. Cannabinoid receptor binding and agonist activity of amides and esters of arachidonic acid. *Mol. Pharmacol.* 46:516–22
 76. Hillard CJ, Edgemond WS, Campbell WB. 1995. Characterization of ligand binding to the cannabinoid receptor of rat brain membranes using a novel method: application to anandamide. *J. Neurochem.* 64:677–83
 77. Adams IB, Ryan W, Singer M, Thomas BF, Compton DR, et al. 1995. Evaluation of cannabinoid receptor binding and in vivo activities for anandamide analogs. *J. Pharmacol. Exp. Ther.* 273:1172–81
 78. Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, et al. 1994. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J. Pharmacol. Exp. Ther.* 270:219–27
 79. Friede E, Mechoulam R. 1993. Pharmacological activity of the cannabinoid agonist anandamide, a brain constituent. *Eur. J. Pharmacol.* 231:313–14
 80. Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA, et al. 1993. Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. *Pharmacol. Biochem. Behav.* 46:967–72
 81. Venance L, Piomelli D, Glowinski J, Ghaume C. 1995. Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. *Nature* 376:590–94
 82. Lake KD, Martin BR, Kunos G, Varga K. 1997. Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension* 29:1204–10
 83. Randall MD, Alexander SP, Bennett T, Boyd EA, Fry JR, et al. 1996. An endogenous cannabinoid as an endothelium-derived vasorelaxant. *Biochem. Biophys. Res. Commun.* 229:114–20
 84. Hanus L, Gopher A, Almog S, Mechoulam R. 1993. Anandamide, an endogenous cannabinoid, binds to the cloned human cannabinoid receptor and stimulates receptor mediated signal transduction. *Proc. Natl. Acad. Sci. USA* 90:7656–66

- lam R. 1993. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J. Med. Chem.* 36:3032-34
85. Barg J, Fride E, Hanus L, Levy R, Matus-Leibovitch N, et al. 1995. Cannabinomimetic behavioral effects of and adenylylate cyclase inhibition by two new endogenous anandamides. *Eur. J. Pharmacol.* 287:145-52
 86. Thomas BF, Adams IB, Mascarella SW, Martin BR, Razdan RK. 1996. Structure-activity analysis of anandamide analogs: relationship to a cannabinoid pharmacophore. *J. Med. Chem.* 39:471-79
 87. Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R. 1997. Structural requirements for binding of anandamide type compounds to the brain cannabinoid receptor. *J. Med. Chem.* 40:659-67
 88. Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, et al. 1996. Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. *J. Med. Chem.* 39:4515-19
 89. Edgemond WS, Campbell WB, Hillard CJ. 1995. The binding of novel phenolic derivatives of anandamide to brain cannabinoid receptors. *Prostaglandins Leukot. Essent. Fatty Acids* 52:83-86
 90. Adams IB, Ryan W, Singer M, Razdan RK, Compton DR, et al. 1995. Pharmacological and behavioral evaluation of alkylated anandamide analogs. *Life Sci.* 56:2041-48
 91. Schmid PC, Krebsbach RJ, Perry SR, Dettmer TM, Maasson JL, et al. 1995. Occurrence and postmortem generation of anandamide and other long-chain N-acylethanolamines in mammalian brain. *FEBS Lett.* 375:117-20
 92. Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, et al. 1996. Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca²⁺-dependent transacylase and phosphodiesterase activities. *Biochem. Biophys. Res. Commun.* 218:113-17
 93. Felder CC, Nielsen A, Briley EM, Palkovits M, Priller J, et al. 1996. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide in brain and peripheral tissues of human and rat. *FEBS Lett.* 393:231-35
 94. Wang PC, Buu NT, Kuchel O, Genest J. 1983. Conjugation patterns of endogenous plasma catecholamines in human and rat. A new specific method for analysis of glucuronide-conjugated catecholamines. *J. Lab. Clin. Med.* 101:141-51
 95. Kuehl FA, Jacob TA, Ganley OH, Ormond RE, Meisinger MAP. 1957. The identification of N-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. *J. Am. Chem. Soc.* 79:5577-78
 96. Bachur NR, Udenfriend S. 1965. Microsomal synthesis of fatty acid amides. *J. Biol. Chem.* 240:1019
 97. Schmid HH, Schmid PC, Natarajan V. 1990. N-acylated glycerophospholipids and their derivatives. *Prog. Lipid Res.* 29:1-43
 98. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, et al. 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons [see comments]. *Nature* 372:686-91
 99. Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, et al. 1996. Transacylase-mediated and phosphodiesterase-mediated synthesis of N-arachidonoyl ethanolamide, an endogenous cannabinoid-receptor ligand, in rat brain microsomes: comparison with synthesis from free arachidonic acid and ethanolamine. *Eur. J. Biochem.* 240:53-62
 100. Mechoulam R, Ben-Shabat S, Hanus L, Ligumdky M, Kaminski NE, et al. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50:83-90
 101. Bisogno T, Sepe N, Melck D, Maurelli S, De Petrocellis L, et al. 1997. Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. *Biochem. J.* 322:671-77
 102. Di Marzo V, De Petrocellis L, Sugiura T, Waku K. 1996. Potential biosynthetic connections between the two cannabimimetic eicosanoids, anandamide and 2-arachidonoyl-glycerol, in mouse neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 227:281-88
 103. Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, et al. 1996. N-arachidonylethanolamine (anandamide), an endogenous cannabinoid receptor ligand, and related lipid molecules in the nervous tissues. *J. Lipid. Mediat. Cell. Signal* 14:51-56
 104. Stella N, Schweitzer P, Piomelli D. 1997. A second endogenous cannabinoid ligand that modulates long-term potentiation. *Nature* 388:773-78

105. Devane WA, Axelrod J. 1994. Enzymatic synthesis of anandamide, an endogenous ligand for the cannabinoid receptor, by brain membranes. *Proc. Natl. Acad. Sci. USA* 91:6698-701
106. Kruszka KK, Gross RW. 1994. The ATP- and CoA-independent synthesis of arachidonylethanolamide. A novel mechanism underlying the synthesis of the endogenous ligand of the cannabinoid receptor. *J. Biol. Chem.* 269:14345-48
107. Cadas H, di Tomaso E, Piomelli D. 1997. Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* 17:1226-42
108. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. 1997. Functional role of high-affinity anandamide transport, as revealed by selective-inhibition. *Science* 277:1094-97
109. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, et al. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384:83-87
110. Deutsch DG, Omeir R, Arreaza G, Salehiani D, Prestwich DG, et al. 1997. Methyl arachidonoyl fluorophosphonate: a potent irreversible inhibitor of anandamide amidase. *Biochem. Pharmacol.* 53:255-60
111. Deutsch DG, Lin S, Hill WA, Morse KL, Salehiani D, et al. 1997. Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. *Biochem. Biophys. Res. Commun.* 231:217-21
112. Muthian SKG, Edgemond WS, Campbell WB, Hillard CJ. 1997. *Diazomethyl arachidonoyl ketone irreversibly inhibits amidohydrolase in isolated rat brain membranes, cultured cells and in vivo.* *Symposium on Cannabinoids*, 1:14. Burlington, VT: Int. Cannabinoid Res. Soc.
113. Voth EA, Schwartz RH. 1997. Medicinal applications of delta-9-tetrahydrocannabinol and marijuana. *Ann. Intern. Med.* 126:791-98
114. Hoffman D, Brunneman DK, Gori GB, Wynder EL. 1975. On the carcinogenicity of marijuana smoke. *Recent Adv. Phytochem.* 9:63-81
115. Wu TC, Tashkin DP, Djahed B, Rose JE. 1983. Pulmonary hazards of smoking marijuana as compared with tobacco. *N. Engl. J. Med.* 318:347-51
116. Sallan SE, Zinberg NE, Frei E 3d. 1975. Antiemetic effect of delta-9-tetrahydrocannabinol in patients receiving cancer chemotherapy. *N. Engl. J. Med.* 293:795-97
117. Sallan SE, Cronin C, Zelen M, Zinberg NE. 1979. Antiemetics in patients receiving chemotherapy for cancer: a randomized comparison of delta-9-tetrahydrocannabinol and prochlorperazine. *N. Engl. J. Med.* 302:135-38
118. Chang AE, Shilling DJ, Stillman RC, Goldberg NH, Seipp CA, et al. 1979. Delta-9-tetrahydrocannabinol as an antiemetic in cancer patients receiving high dose methotrexate. A prospective, randomized evaluation. *Ann. Intern. Med.* 91:819-24
119. Nelson K, Walsh D, Deeter P, Sheehan F. 1994. A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *J. Palliative Care* 10:14-18
120. Tanda G, Pontieri FE, Di Chiara G. 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common m1 opioid receptor mechanism. *Science* 276:2048-50
121. McGregor IS, Issakidis CN, Prior G. 1996. Aversive effects of the synthetic cannabinoid CP55,940 in rats. *Pharmacol. Biochem. Behav.* 53:657-64
122. Parker LA, Gilles T. 1995. THC-induced place and taste aversions in Lewis and Sprague-Dawley rats. *Behav. Neurosci.* 109:71-78
123. Onaivi ES, Green MR, Martin BR. 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* 253:1002-9
124. Harris RT, Waters W, McLendon D. 1974. Evaluation of reinforcing capability of delta-9-tetrahydrocannabinol in rhesus monkeys. *Psychopharmacologia* 37:23-29
125. Leite JR, Carlini EA. 1974. Failure to obtain cannabis-directed behaviour and abstinence syndrome in rats chronically treated with *Cannabis sativa* extracts. *Psychopharmacologia* 36:133-45
126. Corcoran ME, Amit Z. 1974. Reluctance of rats to drink hashish suspensions: free-choice and forced consumption, and the effects of hypothalamic stimulation. *Psychopharmacologia* 35:129-47
127. McGregor IS, Bryant PA, Arnold J. 1995. CP55,940, a synthetic cannabinoid, does not sensitize locomotor activity or cocaine responsiveness with intermittent administration in Wistar rats. *Soc. Neurosci. Abstr.* 21:726
128. Takahashi RN, Singer G. 1981. Cross self-administration of delta 9-tetra-

- hydrocannabinol and D-amphetamine in rats. *Braz. J. Med. Biol. Res.* 14:395–400
129. Sanudo-Pena MC, Tsou K, Delay ER, Hohmann AG, Force M, et al. 1997. Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neurosci. Lett.* 223:125–28
 130. Navarro M, Hernandez E, Munoz RM, del Arco I, Villanua MA, et al. 1997. Acute administration of the CB1 cannabinoid receptor antagonist SR141716A induces anxiety-like responses in the rat. *NeuroReport* 8:491–96
 131. Aberman J, Carriero D, Hill A, Makriyanis A, Salamone J. 1997. *Behavioural effects of cannabinoid agonists and antagonists. Symposium on Cannabinoids*, 1:42. Burlington, VT: Int. Cannabinoid Res. Soc.
 132. Glass M, Faull RLM, Dragunow M. 1993. Loss of cannabinoid receptor in the substantia nigra in Huntington's disease. *Neuroscience* 56:523–27
 133. Richfield EK, Herkenham M. 1994. Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. *Ann. Neurol.* 36:577–84
 134. Welch SP, Thomas C, Patrick GS. 1995. Modulation of cannabinoid-induced antinociception after intracerebroventricular versus intrathecal administration to mice: possible mechanisms for interaction with morphine. *J. Pharmacol. Exp. Ther.* 272:310–21