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From cannabis to cannabinergics: new therapeutic opportunities

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Abstract

The molecular basis of cannabinoid activity is better understood since the discovery of the CB_1 receptor in the mammalian brain and the CB_2 receptor in peripheral tissues. Subsequently, an endogenous CB_1 receptor ligand, arachidonylethanolamide (anandamide), was isolated from porcine brain and shown to be metabolized by the enzyme arachidonylethanolamide amidohydrolase or fatty acid amide hydrolase. Recently, we have characterized a reuptake system for the transport of anandamide across the cell membrane, and have shown that selective inhibition of this transporter is associated with analgesia and peripheral vasodilation. The four cannabinoid system proteins, including the CB_1 and CB_2 receptors, fatty acid amide hydrolase, and the anandamide transporter, are excellent targets for the development of novel medications for various conditions, including pain, immunosuppression, peripheral vascular disease, appetite enhancement or suppression, and motor disorders. During the last decade, numerous selective ligands for each of these proteins were designed and synthesized. Many of these agents serve as important molecular probes, providing structural information about their binding sites, as well as pharmacological tools imparting information about the roles of their targets in physiological and disease states. All of the above compounds that modulate the functions of the endocannabinoid system can be collectively described under the term cannabinergics, regardless of chemical classification or type of resultant pharmacological action.

Keywords: Cannabinoid receptors; Anandamide; Cannabimimetics; Cannabinergics; Therapeutics; CB_1

Abbreviations: AA, arachidonic acid; AAI, aminoalkylindole; AEA, arachidonyl ethanolamide; 2-AG, 2-arachidonyl-glycerol; AIDS, acquired immunodeficiency syndrome; AT, anandamide transporter; CC, classical cannabinoid; DCI, depolarization-induced suppression of inhibition; FAAH, fatty acid amide hydrolase; GPCR, G-protein-coupled receptor; MS, multiple sclerosis; NAPE, *N*-arachidonyl-phosphatidylethanolamine; NCC, nonclassical cannabinoid; PL, phospholipase; SAR, structure–activity relationship; THC, tetrahydrocannabinol.

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1. Introduction

Today, *cannabis* or marijuana is the focus of strong social, legal, and medical controversy over its therapeutic utility. In 1997, two referenda in Arizona and California and, later, others in eight additional states, aimed at providing legal status to marijuana cigarettes for medical purposes. Two licensed single-compound cannabimimetic pharmaceuticals, Marinol® [Dronabinol, (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) from Roxane Laboratories (Columbus, OH, USA)] and Cesamet® [Nabilone developed at Eli Lilly (Indianapolis, IN, USA) now in use in the United Kingdom], are marketed for two indications: the control of nausea and emesis produced by cancer chemotherapy and as appetite stimulants in acquired immunodeficiency syndrome (AIDS)-related anorexia. Both of these agents have proven to be superior to conventional anti-emetics such as perchlorperazine in clinical trials with cancer chemotherapy patients (Breivogel & Childers, 1998).

Beyond this relatively limited medical use of cannabinoids and cannabimimetic agents, the ongoing, long-delayed elucidation of their pharmacology is likely to lead to a wide expansion of their clinical potential and significance. The understanding of cannabinoids and their biology was delayed for two main reasons (Mechoulam & Feigenbaum, 1995). The first was the gum-like, non-crystalline nature of the biologically active terpenoid ingredients of *Cannabis sativa*. (−)-Δ^9-THC, the main active ingredient, was isolated and identified only in 1964 (Mechoulam & Gaoni, 1967). The second reason for this delay in progress was the long-standing scientific misconception that the cannabinoid-induced pharmacological actions are not mediated through specific receptors, but by perturbation of cellular membranes. This hypothesis deterred the pursuit of possible specific cannabinoid binding sites for many years. Due to their high lipophilicity, cannabinoids were paralleled with general anesthetics in terms of their mechanism of action (Paton, 1975). Although cannabinoids were found to clearly perturb membranes (Makriyannis et al., 1987), such effects were never shown to be solely responsible for their biological activity.

2. Cannabinoid receptors

2.1. The CB_1 receptor

In 1988, Devane et al. were the first to demonstrate the existence of specific cannabinoid binding sites in the rat brain. Definitive proof of the existence of the cannabinoid receptor came from Matsuda et al. in 1990 when they isolated the cDNA of a cannabinoid receptor from a rat cerebral cortex cDNA library and expressed it in CHO cells. This receptor was named CB_1, and its 472 amino acid sequence revealed that it is a member of the G-protein-coupled receptors (GPCRs) (Matsuda et al., 1990; Gerard et al., 1991). Its third intracellular loop and the C-terminus are the sites involved in interactions with G-proteins of the $G_{i/o}$ family (Howlett et al., 1998). The C-terminus was found to bind with high affinity to G_i, and the synthetic C-terminus peptide was found to stimulate, by itself, GTPγS binding to G-protein and to inhibit adenylate cyclase (Howlett et al., 1998).

Activation of CB_1 leads to inhibition of adenylyl cyclase and, therefore, to the reduction of cyclic AMP levels. One of the cyclic AMP-dependent cannabinoid effects is the enhancement of voltage-sensitive outwardly rectifying K^+ channels, which occurs as a result of decreased phosphorylation of the K^+ channel protein by A-kinase (Deadwyler et al., 1995). Besides G_i, CB_1 is also shown to be coupled to G_o. Apart from inhibition of adenylyl cyclase, CB_1 utilizes several additional effector systems (intracellular mediators) involving $G_{i/o}$ proteins: the inhibition of N-type Ca^{2+} channels (Mackie & Hille, 1992), the activation of mitogen-activated protein kinase (Bouabula et al., 1995b), and the expression of immediate early genes like *Krox-24* (Bouabula et al., 1995a). Other cannabinoid-induced cellular effects include activation of inwardly rectifying K^+ channels (Pertwee, 1997) and possibly activation of phospholipase (PL)A, PLC, or PLD (Felder et al., 1995).

Different G-proteins or second messengers may couple to CB_1 in different brain regions and may mediate different physiological effects (Howlett et al., 1999). Utilization of diverse effector systems by CB_1 may explain how the

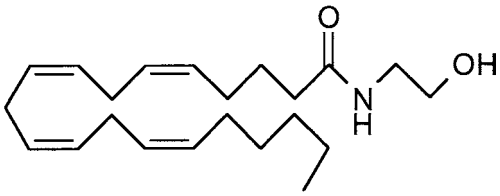

Fig. 1. The structure of AEA.

response to cannabimimetics varies across different types of cells. Understanding which physiological responses are mediated by each of the above intracellular signaling systems is of great significance, and may provide new grounds for the design of selective cannabimimetic agents.

2.2. The CB_2 receptor

Homology cloning revealed the existence of a second cannabinoid receptor, CB_2 (Munro et al., 1993). This receptor shows 44% identity to the total CB_1 and 68% identity within the transmembrane regions. CB_2 is present in the periphery and mainly in tissues of the immune system. Localization of CB_2 in the immune system suggests an immunomodulatory role for this receptor. Thus, CB_2 may be the mediator of the long-known immunosuppressive properties of marijuana.

CB_1 and CB_2 share some common signal transduction pathways, such as inhibition of adenylyl cyclase and stimulation of mitogen-activated protein kinase. However, unlike CB_1, CB_2 has not been shown to affect ion channels (Pertwee, 1997).

2.3. Cannabinoid receptor distribution

CB_1 is a ubiquitous receptor found in the CNS and the periphery, and in both neural as well as non-neural tissues. CB_1 is one of the most abundant GPCRs in the brain (Breivogel & Childers, 1998). As shown by various mammalian brain autoradiographic studies (Herkenham et al., 1990; Gatley et al., 1998), CB_1 density is highest in the basal ganglia, substantia nigra pars reticulata, entopeduncular nucleus, and the external segment of the globus pallidus. Moderately high CB_1 density is found in the putamen, cerebellum, and hippocampus, whereas moderate levels exist in the cerebral cortex. The spinal cord shows a range of moderate densities, while the thalamus and the brain stem contain low to negligible levels.

In the periphery, CB_1 is found in the adrenal glands, bone marrow, heart, lungs, prostate, testes, thymus, tonsils, spleen, lymphocytes, phagocytes, smooth muscle, vascular endothelium, peripheral neurons (e.g., in the gut), kidneys, uterus, and sperm (reviewed by Schuel et al., 1999).

The CB_2 receptor has a more limited distribution, principally in cells associated with the immune system, such as leukocytes, spleen, thymus, and tonsils (various amounts of CB_1 are found in some of these cells as well) (Cabral, 1999). Among the human blood cells, B lymphocytes express the highest levels of CB_2, followed in order by natural killer cells, monocytes, polymorphonuclear neutrophils, T8 lymphocytes, and T4 lymphocytes.

3. The endogenous ligands

The discovery of the cannabinoid receptors and their G-protein-coupled nature strongly suggested the existence of one or more endogenous cannabimimetic ligands that exert their physiological activity upon binding to these receptors. Initial efforts to identify a possible protein (Nye et al., 1988) or other water-soluble endogenous cannabimimetic ligands were unsuccessful (Deadwyler et al., 1995). The hypothesis that such a putative endocannabinoid should be lipophilic, like the classical exogenous cannabinoids, led Mechoulam and co-workers to search for such a ligand in the hydrophobic fractions of porcine brain extracts (Devane et al., 1992). Repetitive fractionations and purifications led to the identification of a substance that bound to CB_1 in a saturable fashion. This compound was the ethanolamide of arachidonic acid (AA) [arachidonyl ethanolamide (AEA)] (Fig. 1). The authors named this brain constituent anandamide from *ananda*, the Sanskrit word for bliss.

Anandamide is found in the human brain at the following levels: 100 pmol/g in the hippocampus, 75 pmol/g in the thalamus, 60 pmol/g in the cerebellum, and 55 pmol/g in the striatum (Martin et al., 1999). The concentration of AEA increases postmortem, especially when the brain is kept at ambient temperature. Furthermore, AEA surges are observed when cerebellar granule cells are treated in hypoxic conditions (Hillard & Cambell, 1997). Although such concentration increases may be artifacts of postmortem

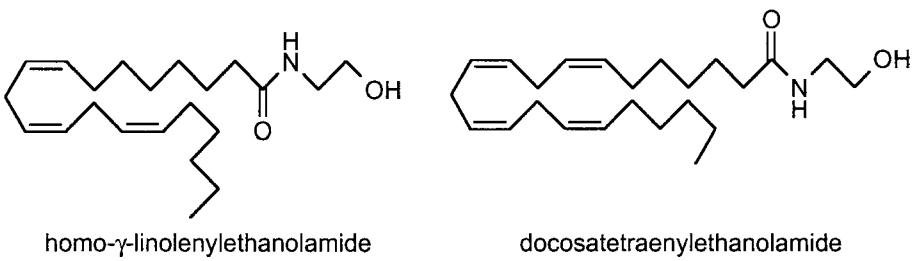

Fig. 2. The structures of two *N*-acylethanolamide endocannabinoids.

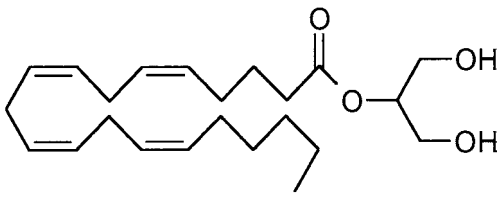

Fig. 3. The structure of 2-AG.

brain damage, they may also occur in living tissue under certain conditions, e.g., hypoxia and brain injury.

In the periphery, anandamide is found in the spleen and heart at ~ 10 pmol/g (Martin et al., 1999). It is also found in rat testes and uterus in concentrations far exceeding those in the brain (Schmid et al., 1997). Very low levels have been detected in serum, plasma, and cerebrospinal fluid, a fact that suggests that anandamide is not hormonal in nature, but is formed at or near its sites of action.

In addition to anandamide, several other endogenous polyunsaturated fatty acid derivatives were also found to act as cannabimimetics. They are now collectively referred to as endocannabinoids. This class of endogenous ligands includes two more fatty acid ethanolamides that bind to CB_1 preparations with similar affinities to that of anandamide (anandamide CB_1 binding affinity: K_i = 39.2 nM, according to Hanus et al., 1993). These are the homo-γ-linolenylethanolamide (CB_1 binding affinity: K_i = 53.4 nM) and 7,10,13,16-docosatetranylethanolamide (CB_1 binding affinity: K_i = 34.4 nM) (Fig. 2).

All three *N*-acylethanolamine endocannabinoids were found to be CB_1 agonists in the mouse *vas deferens* test (Pertwee et al., 1994).

A non-amidic endocannabinoid that is also an AA derivative was first isolated from canine gut and identified as 2-arachidonyl-glycerol (2-AG) (Fig. 3) (Mechoulam et al., 1995).

2-AG was found later also in the brain (Stella et al., 1997) and the spleen (Di Marzo, 1998). It was shown to be released in a Ca^{2+}-dependent manner, and to reach concentrations 170 times higher than those of anandamide in the brain (Stella et al., 1997). Like the other endocannabinoids, 2-AG was shown to produce the typical tetrad of cannabimimetic behavioral effects and to inhibit electrically evoked contractions of mouse *vas deferens* (Mechoulam & Feigenbaum, 1995).

4. Endocannabinoid metabolism

During the past 5 years, there has been considerable progress in our understanding of the physiological pathways that are involved in the synthesis and inactivation of endocannabinoids. Anandamide is currently believed to be formed from membrane phospholipids through a pathway that involves (1) a transacylation of the amino group of phosphatidylethanolamine with arachidonate from the *sn*-1 position of phosphatidylcholine and (2) a D-type phosphodiesterase activity on the resulting N-arachidonyl-phosphatidylethanolamide (NAPE) (Di Marzo et al., 1999a).

2-AG is expected to be biosynthesized by two possible pathways: (1) a PLC-mediated hydrolysis of membrane phospholipids, followed by a second hydrolysis of the resulting 1,2-diacylglycerol by diacylglycerol lipase, or (2) a PLA_1 activity that generates a lysophospholipid, which, in turn, is hydrolyzed to 2-AG by lysophospholipase C (Piomelli et al., 1998).

It is now also believed that endocannabinoids are synthesized within the cell membrane and act on the same or neighboring cells as autocrine or paracrine mediators (Di Marzo et al., 1999a). Experimental evidence to date indicates that anandamide and 2-AG, unlike other classical neurotransmitters, are not stored in vesicles (Cadas et al., 1996, 1997; Di Marzo et al., 1999a). Therefore, it is believed today that anandamide and 2-AG are produced and immediately released from neurons upon demand (Di Marzo et al., 1999a; Piomelli et al., 1998).

The poor water solubility of anandamide must preclude extensive free diffusion in the extracellular space. Thus, it is suggested that anandamide, once cleaved from NAPE, is immediately expelled out of the cell membrane with the assistance of a membrane transporter (such as a P-glycoprotein) (L. Ayotte, R. Picone, and A. Makriyannis, unpublished results) or a lipid-binding protein (such as lipocalin) (Piomelli et al., 1998). Such a lipid-binding protein may also facilitate the diffusion of anandamide through the aqueous extracellular medium to its sites of action.

Anandamide is inactivated in two steps: first by transport inside the cell and second by subsequent intracellular enzymatic hydrolysis. The transport of anandamide inside the cell is a carrier-mediated process, as it was shown to be a saturable-, time-, and temperature-dependent process that involved some protein with high affinity and specificity for anandamide (Beltramo et al., 1997). Although the anandamide transporter (AT) protein is not molecularly characterized, its activity is well characterized and attenuated by specific transporter inhibitors. Reuptake of 2-AG is likely mediated by the same facilitating mechanism (Di Marzo et al., 1999b; Piomelli et al., 1999).

Once anandamide is inside the cell, it is hydrolyzed by the fatty acid amide hydrolase (FAAH) (Desarnaud et al., 1995; Deutsch & Chin, 1993). This enzyme is membrane associated and shows significant specificity for anandamide (Desarnaud et al., 1995; Lang et al., 1999) (Fig. 4).

Less is known about the role and metabolic fate of 2-AG. It is possible that in many tissues, 2-AG is only an intermediate of a signaling pathway that generates 1,2-diacylglycerol and AA, two well-known signaling molecules. In the brain, however, 2-AG may have regulatory roles, since it escapes immediate metabolism and accumulates in response to stimuli-generated Ca^{2+} surges (Stella et al., 1997). This may arise from differences between metabolizing isoenzymes or their levels of expression from tissue to tissue.

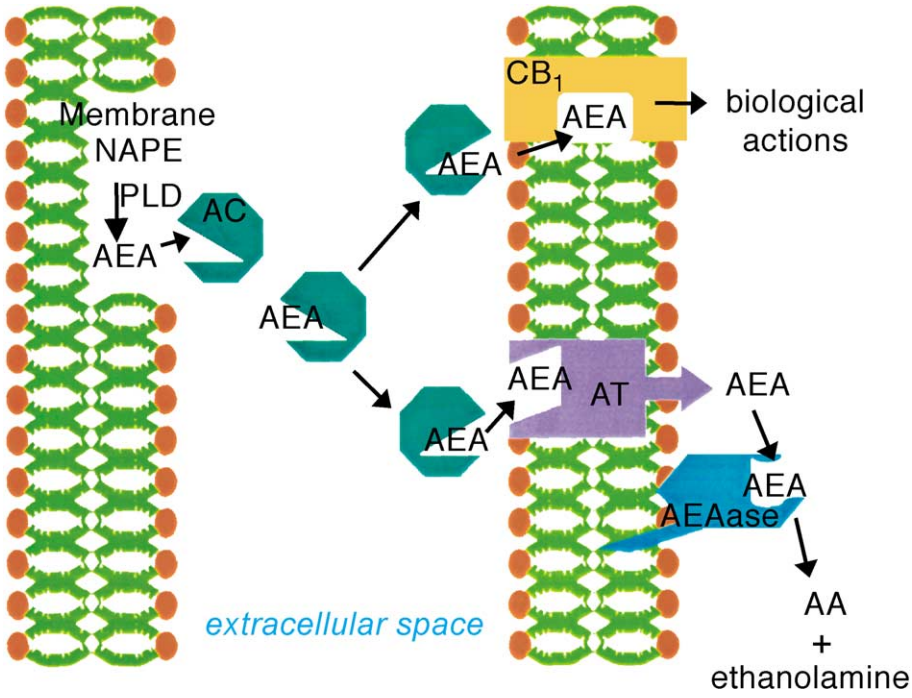

Fig. 4. Anandamide metabolism. AC, anandamide carrier protein; AEA, anandamide; AEAase, anandamide amidase.

Anandamide amidase recognizes and hydrolyzes 2-AG (Goparaju et al., 1999; Di Marzo et al., 1999a, 1999b; Lang et al., 1999). However, there is evidence for the existence of an additional specific hydrolase (monoacylglycerol lipase) that hydrolyzes 2-AG (D. Piomelli and A. Makriyannis, unpublished observation). In addition to this pathway, 2-AG diffuses rapidly into the cell membrane, where it could be either hydrolyzed to AA and glycerol or esterified back to phosphoglycerides (Di Marzo et al., 1999a).

5. The endocannabinoid system

It is apparent from the above discussion that during the last 10 years, a series of important discoveries have unveiled a new, important biological assemblage, the endocannabinoid system. This system, which is evolutionarily well preserved, consists of at least two receptors, each with different localizations and functions; a family of endogenous ligands; and a specific molecular machinery for the synthesis, transport, and inactivation of these ligands. Although new information about this system is emerging, many important questions still remain unanswered. The AT and some of the endocannabinoid metabolic enzymes still need to be cloned. The accomplishment of a highly quantitative and detailed mapping of the endocannabinoid system will provide more information about its physiological roles. The advent of specific cannabinoid receptor antagonists (Pertwee et al., 1995b; Rinaldi-Carmona et al., 1994) has already facilitated cannabinoid research by enabling reversal of the endogenous cannabinoid tone, as well as verification of the interaction of various agents with these receptors. The first inhibitors of anandamide amidase (Boger et al., 2000; Deutsch et al., 1997; Deutsch & Makriyannis, 1997) and its transporter (Pertwee et al., 1995a; Beltramo et al., 1997; Christie & Vaughan, 2001; Wilson & Nicoll, 2001) are becoming important tools in understanding the functions of the endocannabinoid system by producing a hypercannabinoid state.

Pharmacological studies that will further elucidate the role of the endocannabinoid system in physiological and disease states are dependent on the availability of selective agents that interact specifically and selectively with each of the endocannabinoid proteins and in turn, either activate or inhibit them. Therefore, structure–activity relationship

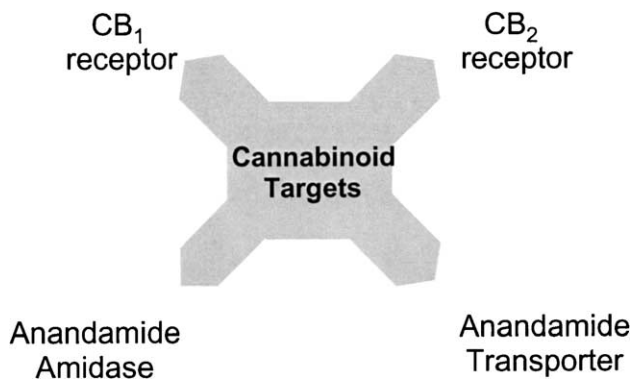

Fig. 5. Cannabinoid targets for drug design.

(SAR) studies on each of these targets and the subsequent identification of differences in their ligand recognition are of great significance, as they can lead to the development of selective cannabinergic agents. In addition, such studies may assist the emergence of new therapeutic agents that act through the endocannabinoid system. This therapeutic potential renders the four characterized endocannabinoid proteins as important, yet not fully exploited, targets for drug design and development (Fig. 5).

6. Major classes of cannabinergic ligands

Based on chemical structure, cannabinergic ligands are classified into five major classes. The term cannabinergic encompasses ligands that act on proteins of the endocannabinoid system, regardless of chemical classification or type of resultant pharmacological activity. Therefore, this general term includes agents that act on the cannabinoid receptors, either as activators or antagonists, as well as molecules that inhibit the FAAH or AT. This is distinct from the term cannabimimetic, which applies only to activators of the endocannabinoid system, or from the term cannabinoid, which characterizes the terpenoid derivatives of *Cannabis*.

Structures of representative members from each of the five chemical classes are shown in Figs. 6–10.

6.1. Classical cannabinoids

Classical cannabinoids (CCs) are tricyclic terpenoid derivatives bearing a benzopyran moiety (Fig. 6). This class includes the natural product (−)-Δ^9-THC and the other pharmacologically active constituents of the plant *C. sativa*.

Many CC analogs have been synthesized and evaluated pharmacologically and biochemically (Razdan, 1986; Mechoulam et al., 1999). The CC structural features that seem to be important for cannabimimetic activity (Makriyannis & Rapaka, 1990) are (1) the phenolic hydroxyl group, which can be substituted by an amino group, but not by a thiol group. In contrast to the traditional CC SAR that considers the phenolic hydroxyl as one of the necessary pharmacophoric groups, analogs lacking it or bearing it in its etherified form retain high receptor-binding affinity, (e.g., analog **2a**), especially for CB_2 (Huffman et al., 1996). (2) The benzopyran ring is not essential for activity. The pyran oxygen can be substituted by nitrogen or it can be eliminated in open ring mono- or bisphenolic compounds. The latter gave rise to the non-CC (NCC) class described in Section 6.2. (3) Neither the double bond nor the 9-methyl group are necessary for activity. (4) The alkyl chain is probably the most essential CC pharmacophoric group. Increased biological activity results from elongating the 5 carbon Δ^9-THC chain to a 7 carbon chain substituted with 1′,1′- (e.g., ***2***) or 1′,2′ dimethyl or with 1′,1′-cyclic moieties (e.g., ***3***, AMG3). Oxygen atoms (ethers) and unsaturation (Papahatjis et al., 1998) within the chain, or terminal halogens, carboxamido, and cyano groups are well tolerated (Khanolkar et al., 2000). (5) An additional pharmacophoric element, the southern aliphatic hydroxyl (Makriyannis & Rapaka, 1990), to produce highly potent classical/NCC hybrids (e.g., **4**, AM919), was developed by Makriyannis and co-workers (Drake et al., 1998), instigated by the SAR of NCCs (see the next section).

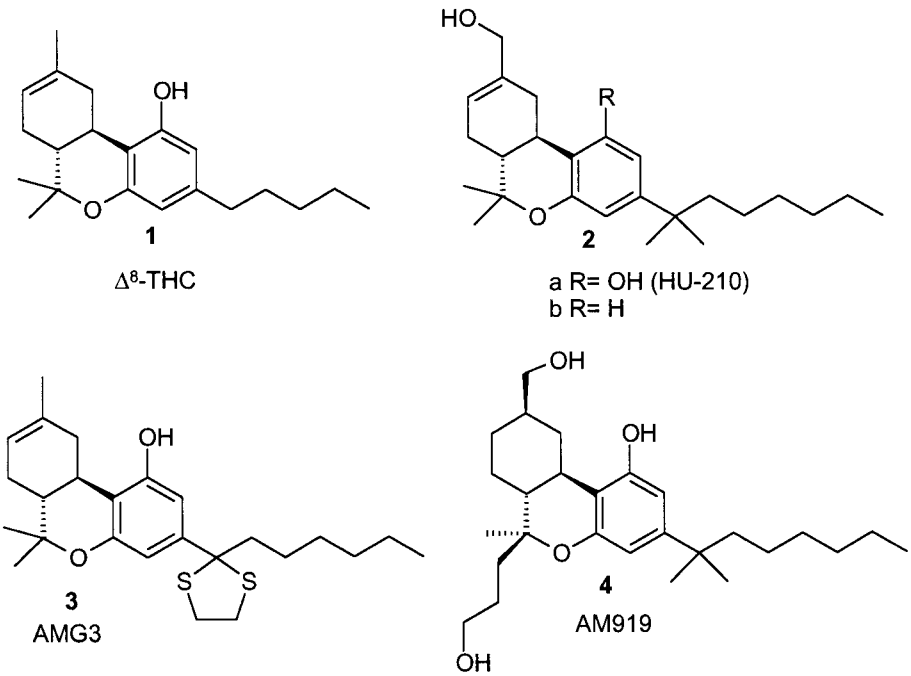

Fig. 6. Structures of representative CCs.

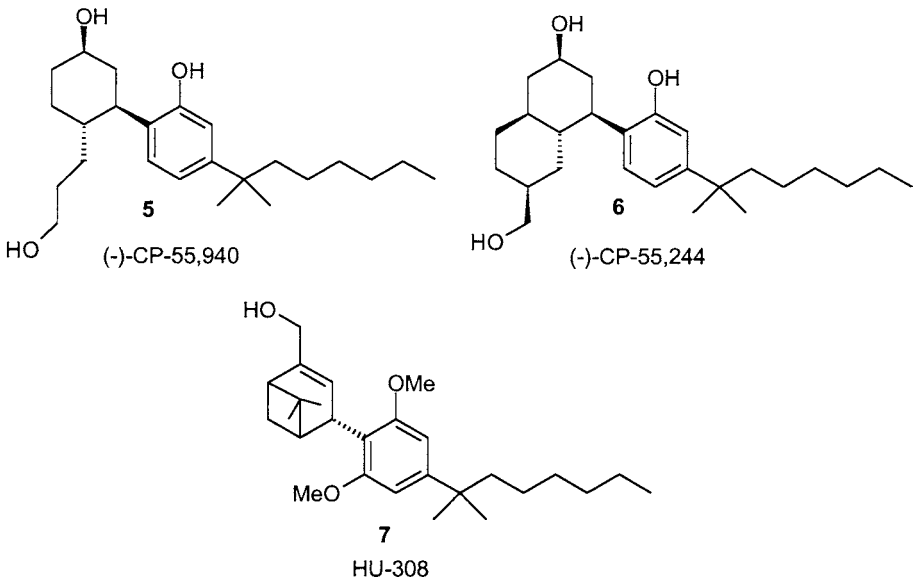

Fig. 7. Structures of representative NCCs.

6.2. Nonclassical cannabinoids

A second class of cannabimimetics was developed at Pfizer (Groton, CT, USA) in an effort to simplify the structure of CCs, while maintaining or improving activity (Johnson & Melvin, 1986; Little et al., 1988). This class includes bicyclic (e.g., **5**) and tricyclic (e.g., **6**) analogs lacking the pyran ring of CCs. These compounds are collectively specified as NCCs (Fig. 7). The crystalline CP-55,940 (**5**) and its tritiated analog show high affinity, efficacy, and stereoselectivity to both cannabinoid receptors, and have been used extensively as pharmacological tools. [^{3}H]CP-55,940 was the key compound that led to the discovery of CB_1 (Devane et al., 1988). The recently discovered CB_2-selective ligand HU-308 (**7**) is another example of such a bicyclic cannabinoid receptor ligand (Hanus et al., 1999).

The structural resemblance of NCCs and CCs, as well as their comparable SARs, indicate that they bind to CB_1 in a similar fashion. The side chain and the phenolic hydroxyl of the NCC are crucial for activity. The hydroxypropyl chain of CP-55,940 is not necessary for activity. However, when present, its stereochemistry is important, with a strong preference for the β relative configuration.

6.3. Aminoalkylindoles

The third chemical class of cannabinergics is that of aminoalkylindoles (AAIs) (Fig. 8). They were developed at Sterling Winthrop (Rensselaer, NY, USA) as potential nonsteroidal anti-inflammatory agents (Bell et al., 1991). These first analogs exhibited antinociceptive properties that eventually were attributed to interactions with the cannabinoid receptors. Compound **8** (WIN55212) is a potent CB_1 and CB_2 agonist, with high stereoselectivity and a slight preference for CB_2. AM630 (**9**), the first CB_2-selective antagonist derived from this class of compounds, was reported from our laboratory in 1994 after long-term efforts for the development of such an inhibitor (Pertwee et al., 1995b). We recently reported Compound **10** (AM1241), a highly CB_2-selective and potent agonist (Malan et al., 2001).

This class of compounds differs from the first two by being considerably less lipophilic and more "drug-like."

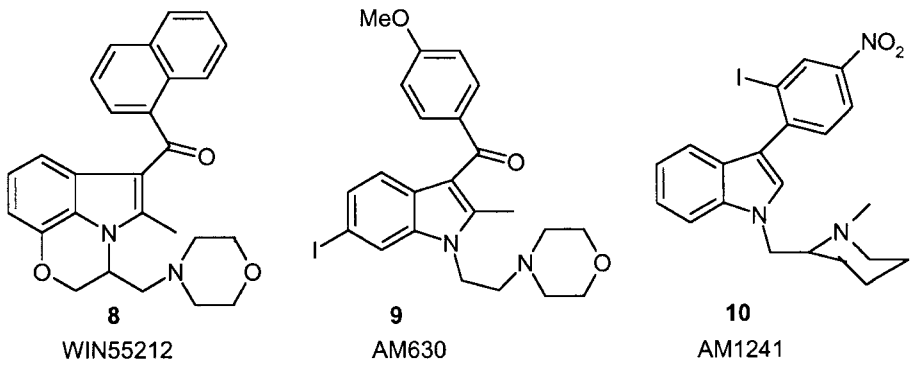

Fig. 8. Representative cannabinergic aminoalkylindoles.

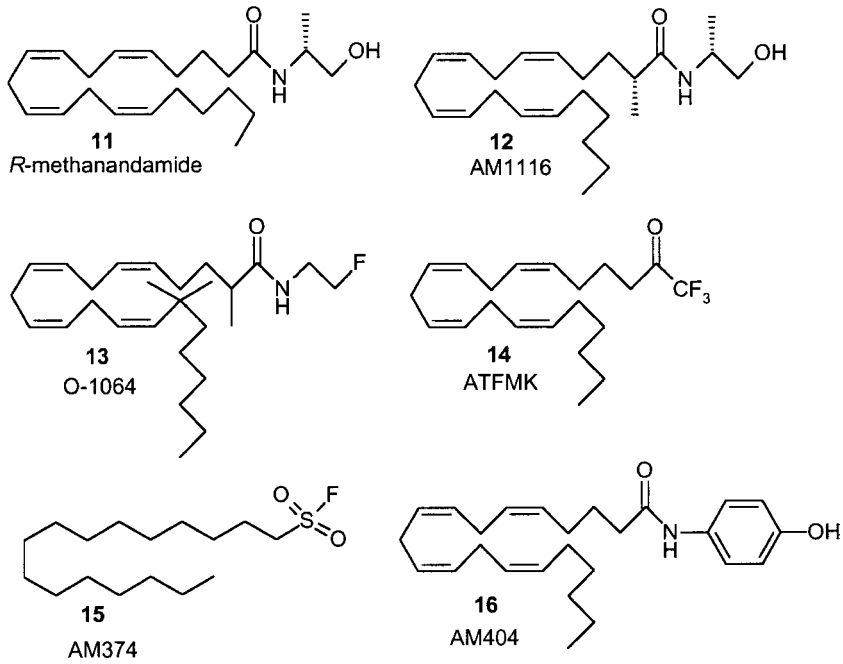

Fig. 9. Structures of representative endocannabinoid analogs.

CB_1 labeling with electrophilic AAIs almost abolished the ability of the receptor to bind to CP-55,940, indicating that AAIs and NCCs (as well as CCs) share at least some points of interactions with CB_1 (Yamada et al., 1996). Several models have attempted to define the pharmacophoric equivalency between the functional groups of AAIs, NCCs, and CCs (Xie et al., 1995; Huffman et al., 1994). Although these three different classes of cannabimimetics show similarities in their binding with CB_1, they differ considerably in the susceptibility of their binding affinities to different Na^+-modulated allosteric receptor states (Houston & Howlett, 1998). They also differ in their affinities to several CB_1 mutants (Chin et al., 1998), as well as in the way they activate the receptor (Houston & Howlett, 1998). These differences may be explained by the existence of more than one ligand-binding motif, or by ligand binding to partially overlapping, but distinct, receptor-binding subsites, or even by induction of different receptor conformational changes upon binding of different ligands (Howlett, 1998). It is proposed that structurally dissimilar ligands may evoke different receptor states (Houston & Howlett, 1998). Therefore, analogs from different cannabinoid ligand classes may evolve as selective pharmacological agents exhibiting only specific cannabimimetic effects.

AAI structural features important for cannabinergic activity are the 3-aroyl moiety and the 1-chain that must contain nitrogen, most often in a heterocyclic ring (piperidino, morpholino). This chain can be constricted as part of a 6-member ring fused to the indole nucleus (D'Ambra et al., 1992).

6.4. Endocannabinoids

The class of the endogenous cannabinoids (endocannabinoids) was discovered in 1992 as molecules with affinity for the cannabinoid receptor produced by mammalian cells

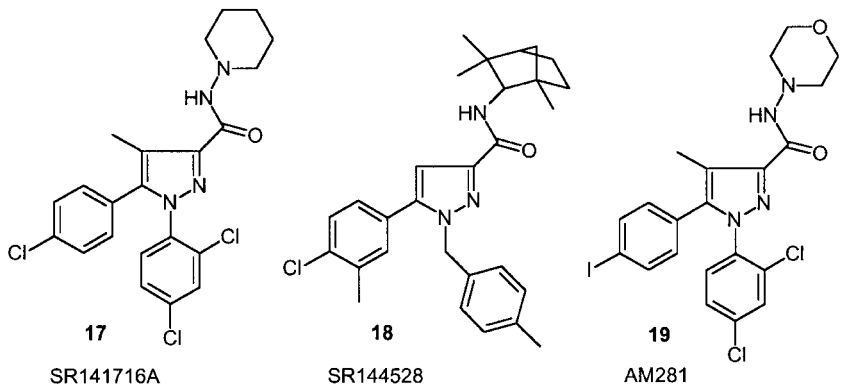

Fig. 10. 1,5 Biarylpyrazole cannabinoid receptor antagonists.

(Devane et al., 1992) (Fig. 9). This class includes lipid molecules, such as fatty acid ethanolamides, monoacylglycerols, and related synthetic analogs. The two prototypes in this class are the ethanolamide of AA, named anandamide, and 2-AG. Its (*R*)-1′-methylated analog AM356 (**11**) shows higher affinity and remarkable metabolic stability (Abadji et al., 1994). This analog, named *R*-methanandamide, has been established as a standard CB_1-selective agonist in the cannabinoid field. The (*R*,*R*)-2,1′-dimethyl anandamide recently was reported to exhibit a 3-fold improved affinity than *R*-methanandamide and significant enantioselectivity (Goutopoulos et al., 2001). Other modifications that result in high CB_1 affinity include the substitution of the hydroxyl group with halogen, or methyl group, and the substitution of the terminal *n*-pentyl chain with the dimethylheptyl chain, reminiscent of potent CC ligands (e.g., **13**, O-1064) (Pertwee, 2000). This class of compounds also includes some fatty acid analogs designed for endocannabinoid targets other than the cannabinoid receptors. For instance, arachidonyltrifluoromethylketone (**14**) and hexadecylsulfonyl fluoride (**15**, AM374) are potent inhibitors of anandamide amidase. AM404 (**16**) was the first inhibitor of the AT that played an important role in the discovery of this protein.

6.5. 1,5 Biarylpyrazoles

The fifth class, that of 1,5 biarylpyrazoles, was developed at Sanofi (Paris, France) in 1994 from a high-throughput screening-generated hit for the cannabinoid receptors (Rinaldi-Carmona et al., 1994) (Fig. 10). Compounds of this class act as cannabinoid receptor antagonists. SR141716A (**17**) was reported simultaneously with AM630 as the first CB_1 antagonist, and has since been used extensively as an important pharmacological tool. This compound shows selectivity for CB_1 and often acts as an inverse agonist rather than a pure antagonist (Pertwee, 2000). SR144528 (**18**), also developed at Sanofi, acts as an antagonist/inverse agonist with selectivity for CB_2. [^{123}I]AM281, a ^{123}I-labeled 1,5-biarylpyrazole synthesized in our laboratory, has served as a useful radioimaging agent in positron emission tomography and single photon emission computed tomography studies (Gatley et al., 1998).

7. Therapeutic potential of cannabinergic agents

Most known cannabimimetics today have very broad effects on organ systems, some of which are still unexplained. The ubiquitous pharmacology of cannabimimetics is one of the reasons why the clinical application of these drugs has not yet reached its full potential. The following sections summarize the effects of cannabinergics on the various physiological systems and the possible therapeutic uses that may emanate from these effects.

7.1. Nervous system

The primary system of cannabimimetic activity is the nervous system. The CB_1 receptor is omnipresent in the brain, especially in areas that control functions known to be affected by cannabimimetics. One of the functions most pronouncedly influenced by cannabimimetics is motor behavior. Catalepsy, immobility, ataxia, and impairment of complex behavioral acts after acute administration of cannabimimetics are manifestations of such motor effects (Pertwee, 1997). In lower doses, they produce the opposite effects. The very dense presence of CB_1 in the cerebellum and the basal ganglia, areas responsible for motor activity, is congruent with these observations. CB_1 agonists enhance γ-aminobutyric acid function in the basal ganglia (Consroe, 1998). Cannabimimetics seem to exert an important modulatory action in basal ganglia output nuclei by inhibiting both inhibitory striatal input, which is tonically inactive, and excitatory subthalamic input, which is tonically active (Sañudo-Peña et al., 1999). The net cannabimimetic effect on motor activity depends on the level of activity of each of these two functions. This may explain the biphasic effect of cannabimimetics on motor behavior.

An important recent discovery has advanced the current understanding of how cannabimimetics are implicated in the control of motor behavior (Giuffrida et al., 1999). It was shown that D_2 activation in the striatum results in release of the endocannabinoid anandamide, which, in turn, seemed to mediate a negative feedback control, counteracting dopamine-induced facilitation of motor activity (Giuffrida et al., 1999).

Because of these effects of cannabinergics on the basal ganglia and subsequently on motor activity, it has been suggested that they may be useful agents in the treatment of motor disorders, such as choreas, Tourette's syndrome (Muller-Vahl et al., 1998), dystonias, and Parkinson's disease (Consroe, 1998). In general, by increasing hypokinetic features in the basal ganglia, CB_1 agonists may alleviate the various hyperkinetic manifestations such as choreic movements that characterize basal ganglia disorders. Direct evidence suggesting the involvement of CB_1 in Huntington's chorea is the extensive loss of CB_1 receptors in the substantia nigra and lateral globus pallidus (Glass et al., 1993). It is still unclear whether these observations are causatives or results of Huntington's disease. However, this finding alone argues that a suitable CB_1 ligand could be potentially useful as a diagnostic agent for this chorea.

Furthermore, the presence of CB_1 in the structures and pathways associated with the pathophysiology of Tourette's syndrome, and especially the functional link between CB_1 and D_1, D_2, also contends that the endocannabinoid system may have some involvement in this disorder as well (Consroe, 1998). In addition, it has been suggested that activation of CB_1 receptors, also due to their link with the dopaminergic system, may reduce dyskinesia produced by levodopa in patients with Parkinson's disease (Brotchie, 1998).

The CB_1 receptors present in the hippocampus, amygdala, and cerebral cortex may be responsible for observations that cannabimimetics are effective against some types of seizures (Consroe, 1998). The anticonvulsant and antispastic effects of cannabinoids are well documented. However, the mechanisms of these effects are still unclear (Nahas et al., 1999). Orally administered cannabimimetics can relieve some of the symptoms of multiple sclerosis (MS) and spinal cord injury, such as muscle spasticity, pain, tremor, nystagmus, and nocturia (Pertwee, 2000). Recent studies (Baker et al., 2000, 2001) have shown that exogenously administered cannabimimetics control spasticity in an MS model. The possible implication of both CB_1 and CB_2 receptors has been suggested. These antispastic effects were also produced indirectly by agents that elevate anandamide levels by inhibiting FAAH (AM374) or AT (AM404). Cannabinoid receptor antagonists blocked these antispastic effects. Respectively, SR141716A and SR1445228, selective CB_1 and CB_2 antagonists/inverse agonists, produced enhanced spasticity when administered alone to the same animal model (Baker et al., 2000). Furthermore, it was evident that endocannabinoids are released during MS to alleviate the spastic effects of the disease (Pertwee, 2000). These findings confirm, at least to some extent, the anecdotal reports that marijuana smoking alleviates the symptoms in MS patients and establishes cannabimimetics as exciting candidates for the development of agents that control spasticity and other abnormalities resulting from some neurodegenerative diseases. Spasticity produced by spinal cord injury may also be controlled by these agents by acting on spinal, as well as on supraspinal, mechanisms (Consroe, 1998). It has been suggested that the effect of cannabimimetics on the release of glutamate in the substantia nigra appears to be the most important supraspinal mechanism of cannabimimetic-induced control of spasticity (Consroe, 1998).

CB_1-mediated inhibition of glutamate release in the hippocampus was also suggested to be the most likely mechanism of the neuroprotective effects of WIN55212 observed in both the global and focal cerebral ischemia animal models (Nagayama et al., 1999). These effects were stereoselective and were blocked by SR141716A. Therefore, cannabimimetics may find potential therapeutic utility in the treatment of disorders resulting from cerebral ischemia, including stroke.

Another neuroprotective activity of cannabimimetics was shown to be associated with the CB_1-mediated inhibition of nitric oxide release from rat microglia cells (Waksman et al., 1999). This study renders cannabimimetics as potentially useful agents in brain injury resulting from inflammatory neurodegenerative processes, especially those involving activation of microglial cells, such as AIDS-encephalitis.

The next most important nervous system-mediated cannabinoid effects are their antinociceptive properties. Compelling evidence suggests that cannabimimetics are effective in the control of acute and chronic pain in a variety of antinociceptive tests in animals (Martin & Lichtman, 1998). Synthetic cannabimimetics have been classified as equal to morphine in potency and efficacy (Walker et al., 1999). The mechanism of the cannabimimetic-induced analgesia is multifaceted and occurs at several levels: (1) directly on spinal cord mechanisms (Walker et al., 1999); (2) in supraspinal mechanisms and specifically in the thalamus and the periaqueductal gray (Walker et al., 1999; Martin & Lichtman, 1998); and (3) in the periphery, possibly involving CB_1-like and CB_2-like receptors (Calignano et al., 1998). Other systems, such as κ- and μ-opiate receptors, as well as spinal noradrenergic mechanisms, seem to be involved in the cannabimimetic-produced analgesia (Walker et al., 1999). Evidence supports that cannabimimetics are effective in animal models of chronic pain, a type of pain that is poorly managed by opioids (Walker et al., 1999). It has also been suggested that CB_1 agonists may be superior to morphine in suppressing pain caused by nerve damage (Pertwee, 2000). This type of pain is signaled by abnormal discharges of Aβ and Aδ fibers, which are much more populated by CB_1 than μ-opioid receptors.

Another category of CNS-mediated cannabinoid effects includes alterations in cognition and memory. Cannabimimetics have been shown to disrupt the performance of working memory tasks at doses lower than those shown to affect the performance of reference memory tasks or to produce the classical tetrad of cannabimimetic effects in mice (Varvel et al., 2001). Conversely, administration of the CB_1 antagonist SR141716A in rats resulted in fewer errors in an eight-arm radial task (Lichtman, 2000). Cannabimimetics have been shown to interfere with the mechanisms of long-term potentiation, a candidate mechanism for learning and memory. They also alter presynaptic release of γ-aminobutyric acid and glutamate from hippocampal neurons (Hampson & Deadwyler, 1998). Hippocampus, a structure rich in CB_1, plays a major role in memory processing, especially by enabling memory retrieval, whereas retrohippocampal areas, with fewer CB_1 receptors, are responsible for memory storage. Hippocampal lesions in rodents impair short-term memory. Several behavioral studies have exhibited that cannabinoids disrupt information processing in the hippocampus, acting as ‘‘reversible’’ hippocampal lesions (Hampson & Deadwyler, 1999). It is suggested that the role of CB_1 in these regions is to switch hippocampal memory circuits in order to regulate storage information (Hampson & Deadwyler, 1998). The role of the cannabinoid system in memory and cognition renders it as a possible target for memory and cognition-enhancing agents. This possibility is strongly supported by some recent advances in understanding the neurobiology of the endocannabinoid system (Wilson & Nicoll, 2001; Christie & Vaughan, 2001; Kreitzer & Regehr, 2001; Ohno-Shosaku et al., 2001). Endocannabinoids were found to be the neurotransmitters responsible for the depolarization-induced suppression of inhibition (DCI) and excitation. DCI enhances memory in the hippocampus. Therefore, drugs that inhibit the metabolism, and especially

the transport of endocannabinoids, are very likely to have a beneficial effect in memory by increasing the levels of endocannabinoids at the sites where DCI takes place (Christie & Vaughan, 2001). Direct cannabinoid receptor agonists flood the endocannabinoid system, resulting in the well-known overall disruptive effect in memory and cognition.

Cannabinoids are long known for their psychoactive and euphoric "high" effects, and have been used for these properties for centuries. Their addictive potential and mechanisms appear to be qualitatively and quantitatively different from those of other drugs of abuse. However, recent studies indicate that cannabimimetics, similarly to addictive drugs, activate the brain reward/reinforcement circuit (ventral tegmental area, nucleous pallidus, and ventral pallidum) and produce reward-related behaviors in laboratory animals (Gardner & Vorel, 1998). Efforts to separate these unwanted effects from the desired ones have had only limited success thus far. This fact, along with the negative social perception of these drugs, are the major reasons that have hampered the development of cannabinergic therapeutics. However, the increasing understanding of the endocannabinoid system presents us with possibilities for the design of selective agents. Indirect activation of this system by increasing endocannabinoid levels only at the sites where they are physiologically produced through inhibition of endocannabinoid catabolism or transport may lead to increased selectivity and fewer undesired side effects than activation of the cannabinoid receptors with direct agonists (Pertwee, 2000). Among the various cannabinoid receptor ligands, endocannabinoids, like anandamide, were shown to have a much lower physical dependence potential (Aceto et al., 1998).

Other well-known, yet not well-understood, central cannabimimetic effects are hypothermia, appetite stimulation, and anti-emetic effects. Cannabimimetic-induced hypothermia is thought to occur by decreasing the thermoregulatory set point through interactions with the relevant hypothalamic centers (Pertwee, 1995). Cannabimimetics also stimulate hunger and food intake in humans and animals, particularly for solid, sweet-tasting foods (Pertwee, 1995; Williams & Kirkham, 1999), an effect that may involve activation of the reward systems (Kirkham & Williams, 2001). For this property, Δ^9-THC (marinol) is clinically used today for the management of AIDS-wasting syndrome (Nahas et al., 1999). The advent of potent and CB_1-selective ligands lacking the CB_2-mediated immunosuppressive properties may present significant advantages over the currently used Δ^9-THC in the treatment of AIDS patients, who are already severely immunocompromised. It is also perceivable that cannabinoid receptor antagonists may be proven effective as appetite suppressants. A study showing that SR141716A, a selective CB_1 antagonist/inverse agonist, suppressed rodent appetite for sucrose, and ethanol serves as proof of the above concept (Arnone et al., 1997). It was shown recently that hypothalamic levels of endocannabinoids are under negative control by leptin (Di Marzo et al., 2001).

A second current clinical indication of cannabimimetics is their anti-emetic and antinausea effects, especially in cancer chemotherapy patients. These effects are mediated above the level of the vomiting reflex and possibly through descending inhibitory connections to the lower brain stem centers (Levitt, 1986).

7.2. Immune system

The discovery of the peripheral CB_2 receptor, which localizes in cells of the immune system, is very likely linked to the well-known immunosuppression of marijuana smokers.

Δ^9-THC was found to decrease host resistance to herpes virus Type 2 in mice and guinea pigs by decreasing both cellular and humoral immunity (Miskin & Cabral, 1985). In vivo and in vitro studies indicate that macrophages are the major targets of cannabinoids. Δ^9-THC inhibits, in a dose-dependent manner, the extrinsic antiviral activity of macrophages (Cabral & Vasquez, 1991). It was also shown that cannabinoids cause morphological changes in macrophages (Cabral & Vasquez, 1991) and affect their phagocytic and spreading ability (Spector & Lancz, 1991).

The involvement of CB_2 (and possibly of CB_1) receptors in the immunosuppressive effects of cannabinoids is not proven yet. The localization of CB_2 in cells of the immune system, and especially in macrophages and lymphocytes, suggests that it serves an immunoregulatory role. The first strong piece of evidence that implicates CB_2 in such a function came from Kaminski et al. (1994), who demonstrated that cannabinoid-induced suppression of humoral immunity was partially mediated through inhibition of adenylyl cyclase by a pertussis toxin sensitive G-protein-coupled mechanism. Involvement of a membrane perturbation mechanism in cannabinoid-induced immunosuppression is also possible, especially in areas exposed to high drug concentrations, such as lung alveolar macrophages of marijuana smokers (Cabral, 1999). In a recent report, 2-AG was found to inhibit production of tumor necrosis factor-α in murine macrophages and in mice (Gallily et al., 2000). The involvement of the cannabinoid system in the regulation of the immune system may argue that cannabinergics potentially could serve as immunomodulatory agents. CB_2 selective agents already exist. However, their clinical potential in some immunomodulatory role will not be realized until the CB_2 physiological functions become better understood. Cannabidiol, a *cannabis* terpenoid ingredient lacking the pyran ring, as well as binding affinity for CB_1 and CB_2, was shown to be an active anti-inflammatory agent in the murine model of arthritis (Pertwee, 2000; Malfait et al., 2000). The molecular basis of this observation remains unknown.

7.3. Cardiovascular system

Cannabinoids reduce platelet aggregation and also produce tachycardia and orthostatic hypotension due to pe-

ripheral vasodilation. A distinct CB_1-like receptor is found in the endothelium of rat mesenteric arteries (Járai et al., 1999). This receptor mediates a remarkable vasodilating effect after activation by any of several CCs, anandamide, or some CB_1-inactive CC-like analogs. This effect is nitric oxide-independent, and it is inhibited by the CB_1 antagonists SR141716A and AM251, and also by cannabidiol. It is possible that exploitation of this new cannabinoid target may lead to new types of hypotensive agents.

7.4. Reproductive system

Cannabinoids produce increased ring and chain chromosomal translocations and morphological abnormalities in mouse sperm, as well as reduction of sperm concentration in humans (Zimmerman et al., 1999). Strong evidence indicates the presence of functional CB_1, or CB_1-like receptors, in human sperm (Schuel et al., 1999). Furthermore, the endogenous cannabimimetic anandamide is produced in human uterus and testes (Schuel et al., 1999). These findings, along with several observations on cannabinoid-induced effects on reproductive functions, suggest that the cannabinoid system may be directly involved in the regulation of sperm production, sperm motility, acrosome reaction, and prevention of polyspermy (Schuel et al., 1999). The endocannabinoid system in the uterus appears to play a fundamental role in embryo implantation and early development. Anandamide inhibits these processes and, therefore, regulation of its levels seems to control the timing of these events (Paria et al., 1995; Paria & Dey, 2000). These findings are also in line with recent clinical observations that correlate the levels of FAAH expression with miscarriages in pregnant women (Maccarone et al., 2000). Further understanding of the endocannabinoid functions in the reproductive system will open perspectives for exploitation of cannabinergics for the treatment of types of infertility or the development of contraceptives.

Cannabimimetics are also shown to affect reproductive and metabolic functions indirectly, by hormonal modulation through the hypothalamic and pituitary regulatory centers. They are found to reduce serum levels of the luteinizing hormone, prolactin, growth hormone, and thyroid-stimulating hormone, and to increase corticotropin (Murphy et al., 1998).

7.5. Eye

Cannabinoids reduce intraocular pressure, probably by directly affecting ocular fluid outflow pathways. The mechanism of this effect is unknown, and its link to cannabinoid receptors has yet to be established (Green, 1999). Marijuana smoking is allegedly helpful to glaucoma patients, and the potential use of cannabimimetics for the treatment of glaucoma is long recognized. New formulation technologies, as well as the advent of less hydrophobic cannabimimetics present us with opportunities to overcome the challenge of local drug delivery to the eye. Recently, AM404 and olvanil, two inhibitors of the AT, were found to decrease the intraocular pressure in rabbits (Laine et al., 2001).

7.6. Respiratory system

Cannabimimetics are known to produce bronchodilation, which is manifested by a marked increase in airway conductance and reduction in airway resistance (Vachon et al., 1973). Although the mechanism of this activity is not known, it probably does not directly involve adrenergic receptors. Possible involvement of CB_{1A} (a CB_1 variant found in the lungs) in the cannabinoid-induced bronchodilation is still unexplored (Shire et al., 1995). Recently, it was shown that anandamide is released in the lungs upon Ca^{2+} stimulation and that it exerts a dual effect on bronchial response: (1) it strongly inhibits capsaicin-evoked bronchospasm and cough. However, (2) it causes bronchoconstriction in vagotomized rodents (Calignano et al., 2000). These effects are mediated by CB_1 receptors present in axon terminals of airway nerves, as they are blocked by SR141716A. This endocannabinoid-mediated control of airway responsiveness may be exploited in the development of new anti-asthmatic agents.

7.7. Gastrointestinal system

Cannabimimetics reduce intestinal motility by a CB_1-mediated inhibitory activity on acetylcholine release from autonomic fibers. 2-AG, an endocannabinoid, was isolated from dog intestine. However, its role there remains unknown (Mechoulam et al., 1995).

8. Conclusions

Cannabinoid research has yielded much information and has taken us toward a better understanding of the molecular mechanisms of cannabinoid action, especially with the discovery of anandamide and 2-AG, two new families of endocannabinoids. Currently, there are multiple known endocannabinoid proteins (at least two receptors, CB_1 and CB_2; an enzyme, FAAH; and a transport protein, AT) as potential therapeutic targets for developing useful medications in the treatment of a multitude of ailments, such as drug addiction, pain, and motor disorders. A number of ligands (receptor-selective agonists/antagonists, inverse agonists, enzyme inhibitors, transport inhibitors) are also available that can serve as important research tools for exploring the endocannabinoid biochemical pathways and their role in the modulation of behavior, memory, cognition, and pain perception. This is significant progress, considering the fact that only about one decade ago the sites of action of cannabinoids had not been identified yet and their molecular mechanism of action was still under question. The future of endocannabinoid research is certainly very exciting and full of promise.

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