THE ENDOCANNABINOID SYSTEM — TIMELINE

Early phytocannabinoid chemistry to endocannabinoids and beyond

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Abstract | Isolation and structure elucidation of most of the major cannabinoid constituents — including Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the principal psychoactive molecule in *Cannabis sativa* — was achieved in the 1960s and 1970s. It was followed by the identification of two cannabinoid receptors in the 1980s and the early 1990s and by the identification of the endocannabinoids shortly thereafter. There have since been considerable advances in our understanding of the endocannabinoid system and its function in the brain, which reveal potential therapeutic targets for a wide range of brain disorders.

The plant Cannabis sativa and its many preparations (for example, marijuana, hashish, bhang and ganja) have been used for millennia for recreation (and at times for the achievement of religious ecstasy) as well as in medicine. In ancient China, cannabis was prescribed (together with other plants, as is customary in Chinese medicine) for numerous diseases, but it was noted that when taken in excess it could lead to 'seeing devils'. In Assyria (about 800 BC), it was named both gan-zi-gun-nu ('the drug that takes away the mind') and azallu (when used as a therapeutic). In India, ancient Persia and medieval Arab societies, cannabis use proceeded along these two divergent routes[1]. In many countries, hemp — a strain of *Cannabis sativa* that does not cause psychoactivity — was grown for its durable fibres. Our present-day society follows a long tradition of recreational, industrial and medical cannabis use.

Cannabinoid discovery — early history

The behavioural effects of cannabis, in several animal species as well as in humans, were observed in the mid-nineteenth century[2] (FIG. 1). These experimental observations led to the first attempts to isolate the active constituents of the plant, as had already been done with other plants that had known neuropharmacological activity — for example, the isolation of morphine. A prize was even awarded in 1855 for the 'successful' accomplishment of this project. However, the first isolation of a plant cannabinoid — named cannabinol (CBN) — was not achieved until the end of the nineteenth century. Its structure was elucidated much later, in the 1930s, by the groups of Cahn and Todd in the United Kingdom and by Adams in the United States, when a further component, cannabidiol (CBD), was isolated; however, its structure could not be elucidated at that time. Although considerable effort was invested on the isolation and the elucidation of the structure of the main psychoactive constituents of cannabis, this goal was not reached at that time. A synthetic compound, $\Delta^{6a,10a}$-tetrahydrocannabinol ($\Delta^{6a,10a}$-THC), showed pharmacological activity that paralleled the activity of cannabis extracts. Therefore, it was assumed that $\Delta^{6a,10a}$-THC was chemically related to the active compounds of the plant (FIG. 2). Much of the early research in this area was done using synthetic $\Delta^{6a,10a}$-THC, which is now known to be considerably less potent than the actual natural product. The chemical and pharmacological work that was carried out until the mid 1940s has been reviewed elsewhere[3–5]. Some $\Delta^{6a,10a}$-THC analogues were even tested in humans. In light of recent media reports about the action of cannabinoids in paediatric epilepsy, it is of interest to note that a derivative of synthetic $\Delta^{6a,10a}$-THC (at doses of 1.2–1.8 mg daily) was administered to a small number of children with epilepsy and showed positive results. Historical cannabis use in medicine over the ages and early chemical investigations are reviewed in REF. 1.

The reasons for the lack of progress were mostly technical. We now know that cannabinoids are present in cannabis as a mixture of many closely related constituents — over 100 — which were difficult to separate using the methods that were available in the nineteenth and early twentieth centuries. As the active constituents of cannabis were not available in pure form, there was very little biological or clinical work done in this area from the late 1940s until the mid 1960s.

By the 1960s, chromatography methods were well developed for the isolation of pure compounds from mixtures and the availability of novel spectrometric methods meant that the elucidation of the structure of these compounds was possible. Indeed, many cannabinoids were isolated, including Δ^9-THC, which was reported by Gaoni and Mechoulam in 1964 (REF. 6) (FIG. 2). Their structures were mainly elucidated using NMR, which was a modern method at the time. Several total syntheses of these compounds have been reported and most cannabinoids are now available as both natural and synthetic products. The chemical work until the mid 1970s is reviewed in REF. 7.

The next step in cannabinoid research was the elucidation of the metabolism of Δ^9-THC and later of CBD. The major metabolic pathway of Δ^9-THC is hydroxylation, which leads to the formation of an active metabolite, followed by its further oxidation to an inactive acid, which then binds to a sugar molecule. These acid-derived metabolites are stored in fatty tissues and are slowly released[8]. Indeed, the major final Δ^9-THC metabolite (a carboxylic acid that is present as a glucuronide) can be detected in human urine for several weeks after cannabis use (FIG. 2).

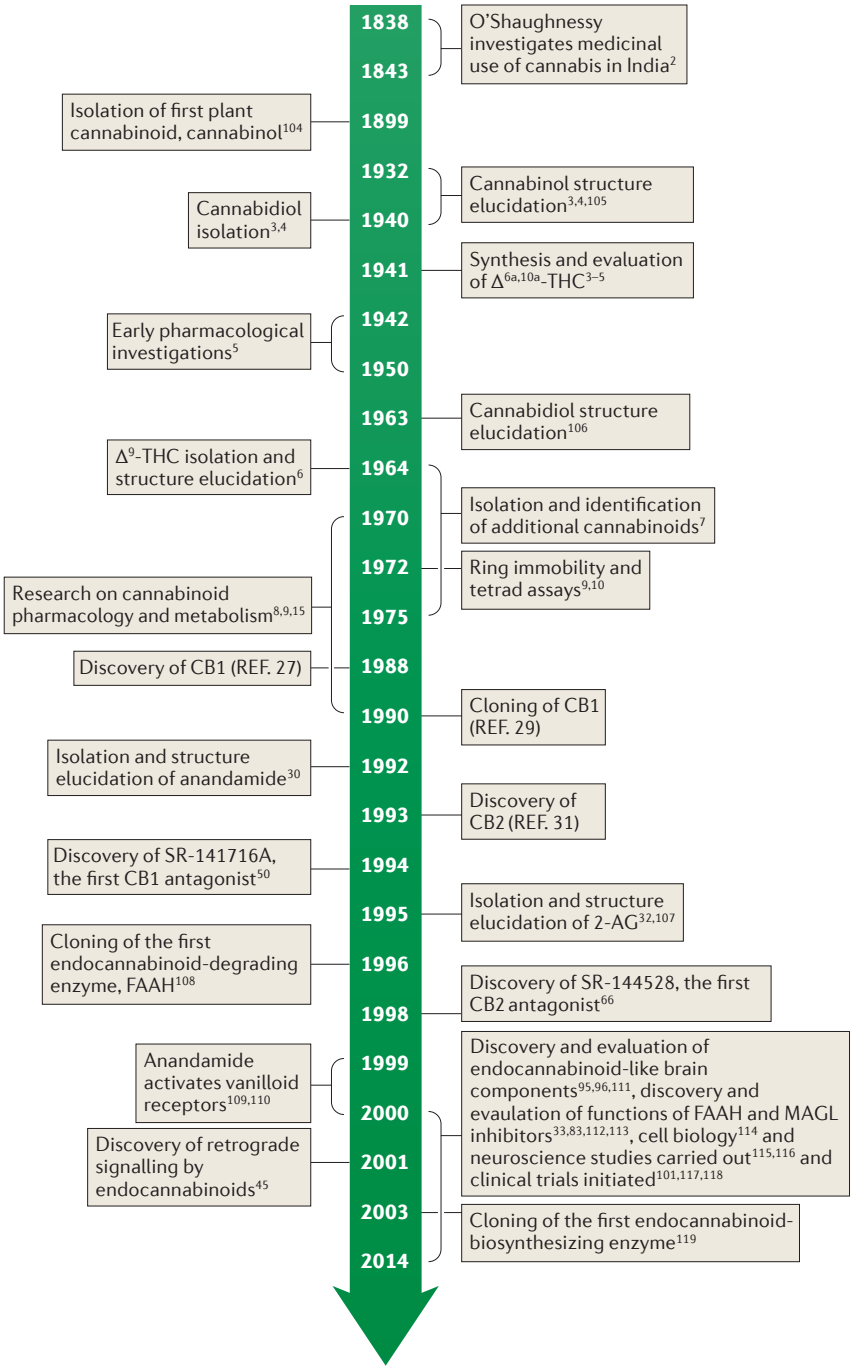


Figure 1 | **Cannabinoid and endocannabinoid research — a timeline.** Almost all early research was devoted to clarification of cannabinoid chemistry[3,4,104,105], and pharmacology was mainly done using synthetic compounds[5]. Following the isolation and structure elucidation of the plant cannabinoids, particularly of cannabidiol[106] and of Δ9-tetrahydrocannabinol (Δ^9-THC)[6], pharmacological and physiological work was initiated[8,9,15]. The identification of cannabinoid receptors[24,29,31], of endogenous cannabinoids[30,32,107] and of receptor antagonists[50,66] made possible extensive pharmacological and neurobiological research leading to cloning of the anandamide-degrading enzyme fatty acid amide hydrolase (FAAH)[108], the discovery of retrograde signaling by 2-arachidonoyl glycerol (2-AG)[45], the discovery of allosteric sites on cannabinoid receptor 1 (CB1)[33], the discovery that endocannabinoids bind to receptors other than CB1 and CB2 (REFS 109–111), the discovery and evaluation of endocannabinoid-like molecules in the brain[95,96] and the discovery and function of inhibitors of the endocannabinoid-degrading enzymes[112,113]. Cell biology[114] and neuroscience[115,116] investigations were also carried out, and clinical trials were initiated[101,117,118]. Cloning of DAG lipase was also reported[119].

Early neuropharmacology

The advances in the chemistry of plant and synthetic cannabinoids led to renewed interest in their neuropharmacology. Loewe[5] had found that cannabis extracts (presumably containing high levels of what is now known to be Δ^9-THC and additional phytocannabinoids) can induce catalepsy in mice and that CBN can also produce this effect, albeit much less potently than the impure THC isolated from the resin. It was these findings that prompted the development by Pertwee[9] in 1972 of a quantitative *in vivo* assay for psychotropic cannabinoids, known as the ring test, in which the proportion of time that a mouse placed across an elevated horizontal ring remains immobile or cataleptic is measured over a 5 minute period[9]. Martin[10] later used this assay, along with three other bioassays, in what came to be known as the 'mouse tetrad assay'[10]. These other assays provide measures of cannabinoid-induced hypokinesia, hypothermia and antinociception in mice, using a tail flick or hot plate test. The mouse tetrad assay is a useful *in vivo* screen for psychotropic cannabinoids, all of which, in contrast to many other types of drugs, generally show similar potency in all four of these bioassays. It was also discovered in the 1940s that cannabinoids can elicit central excitant activity in rabbits and mice and corneal arreflexia in rabbits, and that some phytocannabinoids, particularly CBD, can prolong barbiturate-induced sleep by a mechanism that was subsequently discovered to involve the inhibition of certain cytochrome P450 (CYP) enzymes[10].

Following its identification as the main psychoactive constituent of cannabis, Δ^9-THC attracted particular attention[10,11]; for example, results obtained from several investigations on humans indicated that when Δ^9-THC was taken orally or intravenously or when it was inhaled in smoke, it showed substantial potency at producing psychological changes similar to those reportedly experienced in response to recreationally consumed cannabis[11]. A few other phytocannabinoids, such as CBN, were found to induce cannabis-like effects in humans with low potency (an exception being Δ^8-THC but there is usually very little Δ^8-THC in cannabis)[11].

It is noteworthy that one synthetic analogue of Δ^9-THC, nabilone (Cesamet; Valeant Pharmaceuticals North America) was approved in 1981 as a medicine for the suppression of the nausea and vomiting that is produced by chemotherapy[12]. Synthetic Δ^9-THC, dronabinol (Marinol; Solvay Pharmaceuticals, Inc) subsequently entered

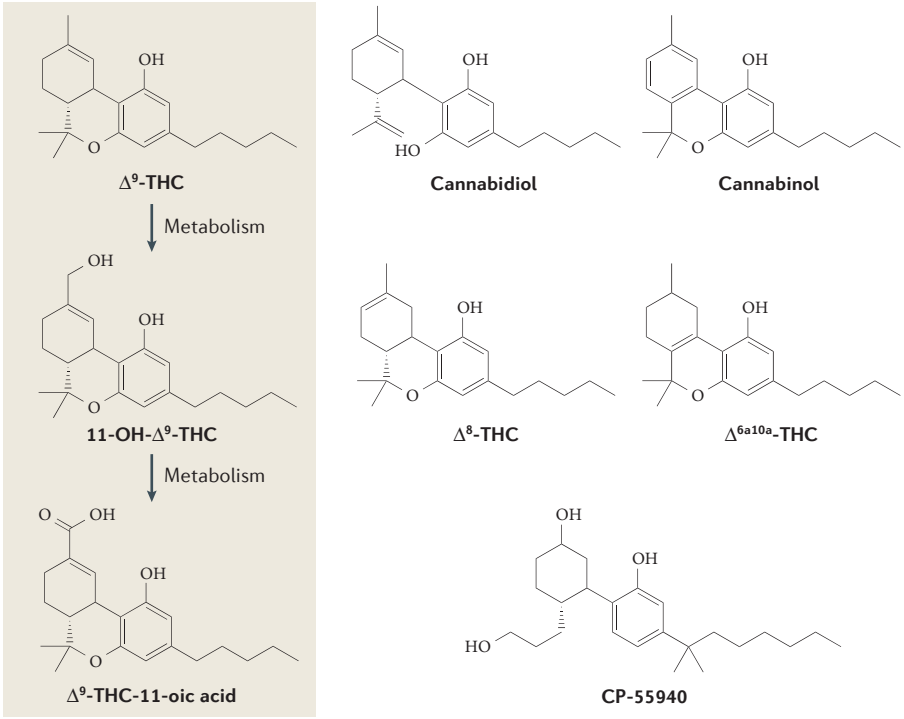


Figure 2 | **A major metabolic pathway of Δ⁹-THC and the structures of some plant and synthetic cannabinoids. a** | The major psychoactive cannabis constituent, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), is first metabolized by enzymatic hydroxylation to produce psychoactive 11-hydroxy-Δ⁹-THC (11-OH-Δ⁹-THC) and then by enzymatic oxidation to non-psychoactive Δ⁹-THC-11-oic acid, which is stored in fatty tissues as a glucuronide and is slowly released. The glucuronide may be detected in the urine for several weeks after a single cannabis use. **b** | The structures of some plant and synthetic cannabinoids. Δ⁹-THC, the plant constituents cannabinol and Δ⁸-THC, and synthetic $\Delta^{6a,10a}$-THC and CP-55940 cause cannabis-type psychoactivity, wherease cannabidiol does not.

the clinic as a licensed medicine, in 1985 as an antiemetic and in 1992 as an appetite stimulant[12]. Claims from patients that cannabis can ameliorate unwanted symptoms of multiple sclerosis also encouraged the development of the cannabis-based medicine naviximols[12] (Sativex; GWPharma), which contains both Δ⁹-THC and the non-psychoactive CBD; this was first licensed as a medicine in 2005 in Canada for the relief of pain experienced by adult patients suffering from multiple sclerosis or advanced cancer, and subsequently as a medicine to ameliorate spasticity caused by multiple sclerosis[12].

Discovery of the cannabinoid receptors

Although a considerable amount of pharmacological work was done on the activity of Δ⁹-THC, its mechanism of action was not elucidated for more than 20 years after its identification. Indeed, it was originally thought that the mode of action of Δ⁹-THC was nonspecific in nature and that it might involve interactions with lipid membranes. However, although the stereospecificity of the action of Δ⁹-THC and related synthetic cannabinoids[13,14], as well as pharmacological studies, in humans and animals had suggested a putative cannabinoid receptor[15,16], it was not until the 1980s that evidence for a protein receptor was sought.

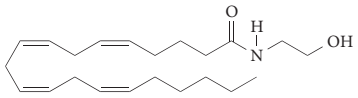
As the family of known G proteins expanded in the late 1970s and early 1980s, so did the list of receptors for hormones and neurotransmitters to which they could couple. Agonists of opioid, muscarinic, cholinergic and α-adrenergic receptors resulted in inhibition of G_s-stimulated adenylyl cyclase[17–19], and functional homology with these neuromodulators led to the discovery that cannabinoids also inhibited this enzyme[20–22] by a pertussis toxin-sensitive mechanism[23]. This clearly indicated that the cannabinoid receptor was a G protein-coupled receptor (GPCR).

From the structure–activity relationship (SAR) established using cannabimimetic compounds from Pfizer Central Research, the Howlett laboratory identified CP55940 (FIG. 2) as a highly potent cannabinoid analogue and, in 1988, reported the determination and characterization of a cannabinoid receptor from the brain for which the criteria for a high-affinity, stereoselective receptor in brain tissue had been fulfilled[24]. Competitive displacement of [³H]CP55940 from its target in rat brain membranes by cannabinoid agonists was enantioselective and followed the order of potency for both G_i-mediated inhibition of adenylyl cyclase as well as antinociception in several rodent models[24–27]. Later, signal transduction assays were used to ultimately deorphanize a 7-transmembrane receptor now known to be the cannabinoid receptor 1 (CB1; also known as CNR1)[28,29].

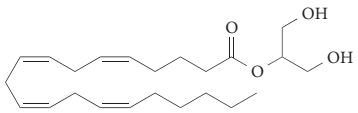
Discovery of endocannabinoids and CB2

Receptors are mostly activated by endogenous molecules, and therefore, there was a strong reason to look for endogenous cannabinoids. As Δ⁹-THC and its related compounds that bind to the CB1 are lipids, it was reasonable to assume that any endogenous cannabinoids would also be lipids. In order to isolate putative endogenous cannabinoid compounds, the ability of porcine brain extracts to displace a novel, highly potent radiolabelled cannabinoid probe, [³H]HU-243, bound to CB1 was tested in the Mechoulam laboratory. The fractions that inhibited the binding of [³H]HU-243 to the cannabinoid receptor were purified by a series of chromatographies, which ultimately led to the generation of a minute amount of a single compound, an amide of arachidonic acid — arachidonoyl ethanolamide — which was named anandamide; this was the first endocannabinoid to be identified[30]. The structure of anandamide (FIG. 3) was established by mass spectrometry, NMR spectroscopy and by its synthesis[30]. Anandamide was found to have inhibitory activity that was equivalent to that of Δ⁹-THC and was subsequently shown to have cannabimimetic activity as it inhibited the twitch response of isolated mouse vasa deferentia[30].

In the meantime, a second receptor, CB2 (also known as CNR2), had been identified by sequence homology[31] and was presumed to be mainly present in the periphery; therefore, a search for a 'peripheral' endogenous agonist was initiated. Using the same techniques that were used to isolate anandamide, it was possible to isolate an ester of arachidonic acid — 2-arachidonoyl glycerol (2-AG)[32] — from canine intestines (FIG. 1). This compound was unexpectedly found to bind CB1 and CB2 and to inhibit adenylyl cyclase with a potency similar to that of Δ⁹-THC. 2-AG also shared the ability of Δ⁹-THC and anandamide to inhibit electrically evoked contractions of isolated mouse vasa deferentia; however, it was less potent than Δ⁹-THC[32]. Following administration



Arachidonoyl ethanolamide



2-Arachidonoyl glycerol

Figure 3 | **Structures of the main endocannabinoids, anandamide and 2-AG, which bind to CB1 and CB2 endocannabinoid receptors.** Arachidonoyl ethanolamide (also known as anandamide) and 2-arachidonoyl glycerol (2-AG) are hydrolysed to arachidonic acid by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Blocking these enzymes with various synthetic compounds leads to increased levels of these endocannabinoids.

to mice, both anandamide and 2-AG caused the typical tetrad of effects produced by Δ^9-THC: antinociception, immobility, reduction of spontaneous activity and lowering of rectal temperature.Although a few additional endocannabinoids have been reported, none of them has been confirmed as a natural endocannabinoid.

Anandamide is a partial agonist for CB1 and CB2 and shows less relative intrinsic activity (also known as relative intrinsic efficacy) and affinity for CB2 than for CB1. 2-AG shows greater potency and efficacy than anandamide as a CB1 agonist and greater potency than anandamide as a CB2 agonist[33]. In addition, it has been found that both endocannabinoids interact with certain non-CB1 and non-CB2 receptors and ion channels[33]. In the past few years, lipoxin A4 and a new family of peptides (known as pepcans) have been reported to target CB1 as allosteric modulators[34,35] and the peptide hemopressin, which is a putative brain constituent, has been found to lower pain via action on a cannabinoid receptor[36].

Synthesis of cannabinoid analogues that have high affinity and specificity for CB2 was achieved in the mid to late 1990s[37,38] and led to the discovery of the role of CB2 in immunosuppression, neuroprotection and neuropathic and inflammatory pain. This consequently led to considerable interest in developing and investigating CB2-selective agonists[39–43].

Both anandamide and 2-AG are synthesized on demand, often in response to increased concentration of intracellular calcium[44], and it is now generally accepted that one important role of these endocannabinoids, although possibly only of 2-AG, is to function as retrograde synaptic messengers that can prevent the development of excessive neuronal activity in the central nervous system and thereby contribute to the maintenance of homeostasis in both health and disease[45]. Thus, there is good evidence that neurotransmitters, such as glutamate, produce postsynaptic increases in the concentration of intracellular calcium in a manner that can induce postsynaptic biosynthesis and release of anandamide or 2-AG into the synapse. In turn, this induces subsequent endocannabinoid-induced activation of presynaptic CB1, which causes an inhibition of the neuronal release of glutamate, γ-aminobutyric acid or other neurotransmitters in brain areas that include the cerebral cortex, hippocampus, ventral tegmental area, substantia nigra, hypothalamus and cerebellum[46–48]. There is also evidence that, when produced postsynaptically in response to the activation of postsynaptic metabotropic glutamate receptor 5 (MGLUR5) , anandamide activates postsynaptic transient receptor potential cation channel subfamily V member 1 (TRPV1) channels[48]. It is also noteworthy that results obtained from *in vivo* experiments with rats suggest that retrograde 2-AG signalling that is triggered by the activation of MGLUR5 can suppress pain sensitivity[49]. The endocannabinoid retrograde transport mechanism and modulation of synaptic transmission have not yet been fully elucidated[46–48].

Search for antagonist ligands

The holy grail for cannabinoid synthetic chemists was an antagonist that could block the effects of Δ^9-THC. It seems quite unusual that no natural product or structurally related analogue emerged to block the cannabinoid receptors. Before the advent of gene knockout techniques, it was difficult to establish whether a pharmacological effect was mediated by a receptor if a selective antagonist for that receptor had not been developed. Thus, one can imagine the excitement generated at an International Cannabinoid Research Society meeting in 1993 when a team of researchers from the French pharmaceutical company Sanofi Recherche announced their discovery of an antagonist for CB1, SR141716A[50]. This compound was radiolabelled to investigate receptor pharmacology[51] and was soon modified to develop the first ligands for *in vivo* imaging[52]. The discovery of an antagonist (SR141716A), which was in fact subsequently identified as an inverse agonist, helped to characterize additional cellular signalling pathways for CB1 (REFS 50, 51, 53–55). More importantly, an antagonist could finally be used to identify animal behaviours that were truly due to CB1 activation[56–58]. Indeed, the syndrome of 'dependence' on cannabinoid agonists was first shown in an animal model after precipitated withdrawal using SR141716A[59,60]. Within a short period of time, industrial laboratories and academic research groups reported the synthesis of additional CB1 antagonists and inverse agonists[61–64].

The first CB2-selective antagonists AM630 (also known as iodopravadoline) and SR144528 emerged in the mid 1990s[65,66] and increased the ability to discern novel actions that could be attributed to CB2, including actions observed in liver Kupfer cells[67], microglial cells and astrocytes[68,69] and in the gastrointestinal system[70], among others. Since that time, there has been considerable progress towards the development of highly selective and potent CB2 antagonists[41,71].

SR141716A (also known as rimonabant) is used therapeutically for the treatment of obesity-related metabolic syndrome components, including dyslipidaemia and diabetes[72–74]. SR141716A was marketed in Europe but failed to gain approval from the US Food and Drug Administration. As might be predicted, a drug that blocks CB1 neuromodulation at synapses for the major stimulatory (in the case of glutamate) and inhibitory (in the case of GABA) transmitters throughout the brain would be likely to produce multiple 'off-target' effects. One such side effect, which was reported in 2009, was an increase in reported signs of depression in vulnerable individuals treated with SR141716A[75,76]. It could be argued that the benefit to risk ratio in a morbidly obese patient population might mitigate the concerns about depression. However, the drug was withdrawn from the market and similar analogues from other pharmaceutical companies were taken out of the development pipeline. Nevertheless, the development of SR141716A by Sanofi–Aventis can be considered to be a major contributor to our understanding that CB1 is present and functional in tissues such as adipose, liver and pancreas under pathological conditions of high-fat diet or obesity[77]. This new understanding of the role of CB1 in metabolic regulation has inspired the search for novel antagonists that fail to gain access to the brain[78,79]. An alternative clinical strategy would be to screen for individuals who might be most susceptible to the limbic effects of CB1 antagonists before selecting a treatment modality[80].

Endocannabinoid neuropharmacology

The discovery that anandamide and 2-AG are endocannabinoids prompted research to identify the biochemical processes that are responsible for both their biosynthesis and their metabolism. This research showed that these two endocannabinoids are synthesized 'on demand' rather than stored, and it identified biosynthetic and metabolic pathways for both of them[81–83]. Thus, it has been discovered that 2-AG is formed from diacylglycerol (DAG) in a process that is catalysed by *sn*1-specific DAG lipase-α and lipase-β, and that the main biosynthetic pathway for anandamide involves the formation of *N*-arachidonoyl phosphatidylethanolamine (NAPE) from phosphatidylethanolamine and phosphatidylcholine, which is catalysed by an as yet uncharacterized calcium-dependent transacylase enzyme. This is then followed by the conversion of NAPE to anandamide in a single step that is catalysed by NAPE-selective phospholipase D and/or in two or three steps that are catalysed by other enzymes. It has also been found that, following their release, anandamide and 2-AG are mainly metabolized to arachidonic acid, the major metabolizing enzymes being fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerol lipase (MAGL) for 2-AG[81,82]. Other endocannabinoid-metabolizing enzymes include FAAH-2 for anandamide, α,β-hydrolase domain-containing 6 (ABDH6) and ABDH12 for 2-AG, and cytochrome P450 enzymes, lipoxygenases and cyclooxygenase 2 for both of these endocannabinoids[81,82]. The physiological relevance of the lipoxygenase and cyclooxygenase derivatives of anandamide and 2-AG is not yet clear. It is also noteworthy that anandamide and 2-AG can undergo cellular uptake following their release, although whether this process is mediated by a transporter is currently unclear[81,82].

It is now recognized that, although engineering exogenous cannabinoids provided insights into receptor usage and linked functional events, the intracellular and extracellular actions and fate of endocannabinoids versus those of exogenously introduced cannabinoids may differ and have different physiological consequences [33,44]. It is also recognized that many cannabinoid receptor ligands also interact with a wide range of non-cannabinoid receptor targets and that, irrespective of whether they are endogenous, synthetic or plant cannabinoids, the pharmacological profiles of these compounds often vary considerably from each other[33,44].

The endocannabinoid receptors, the endocannabinoids and their biosynthetic and biodegrading enzymes constitute what has come to be known as the endocannabinoid system, the discovery of which prompted a search for its physiological and pathophysiological roles. This search revealed that there are several disorders in which endocannabinoids are released to their receptors in an 'autoprotective' manner that ameliorates unwanted effects of these disorders[82–84]. It also raised the possibility that increasing extracellular levels of a released endocannabinoid by inhibiting metabolizing enzymes such as FAAH or MAGL, or by inhibiting the cellular uptake of anandamide, might prove to be an effective therapeutic strategy to manage some of these disorders, which include multiple sclerosis, Parkinson's disease, schizophrenia, hypertension, inflammatory bowel diseases, pruritus, Alzheimer's disease, depression, obsessive compulsive disorder and cancer[82–84].

The discovery of the endocannabinoid system also led to a reinvestigation of the interactions of plant and synthetic cannabinoids with this system and other biochemical entities. As a result, evidence has emerged that Δ^9-THC targets receptors other than CB1 (REFS 85–87). For example, at submicromolar concentrations, Δ^9-THC has also been found to have several effects: first, it has been found to activate CB2, albeit with less efficacy than it activates CB1 (REF. 88); second, it has been found to activate the deorphanized GPCRs GPR18 (REF. 89) and GPR55 (REF. 33), the cation channels TRPA1 and TRPV2 (REFS 90) and the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ)[33]; third, it has been found to block the activation both of 5-hydroxytryptamine 3 (5HT3) ligand-gated ion channels[33,85] and of TRPM8 cation channels[90]; and, last, it has been found to enhance the activation both of α1 subunits and α1β1 dimers of human glycine ligand-gated ion channels and of native glycine receptors in rat isolated ventral tegmental area neurons[33]. There have also been reports that submicromolar concentrations of Δ^9-THC can inhibit the enzyme lysophosphatidylcholine acyl transferase[11], that it can increase the activity

Glossary

Affinity
The potency with which a compound binds to a particular receptor; the higher the affinity of the compound, the lower the concentration at which it achieves a given level of receptor occupancy.

Agonists
Compounds that can activate pharmacological receptors; a full agonist is more potent than a partial agonist and so usually produces a greater maximum functional response.

Allosteric modulators
Drugs that can act on an allosteric site of a receptor to increase or to reduce the ability of an agonist or an inverse agonist to induce a functional response when it targets a different (orthosteric) site on the same receptor.

Antagonist
A compound that can bind to, but cannot activate, a receptor by targeting its orthosteric site and that can therefore prevent both drug-induced agonism and drug-induced inverse agonism at this receptor.

Antinociception
Another term for pain relief.

Apoptosis
A process of programmed cell death that usually has advantageous consequences.

Catalepsy
A condition that is characterized by immobility and muscular rigidity.

Endocannabinoid
An endogenous compound that can directly activate or block cannabinoid CB1 and/or CB2 or that can act as a positive or negative allosteric modulator to increase or to reduce responses of CB1 and/or CB2 to direct agonists or inverse agonists.

G protein-coupled receptor
(GPCR). A seven-transmembrane domain receptor that induces G-protein-mediated activation of intracellular signal transduction pathways when occupied by an agonist.

Hashish
A cannabis-derived preparation that consists mostly of dried cannabis resin.

Hypokinesia
A condition that is characterized by decreased bodily movement.

Inverse agonist
A compound that binds to a receptor in a manner that induces a pharmacological response opposite to the response that is induced by an agonist for the same receptor.

Relative intrinsic activity
The relative ability of drug–receptor complexes to produce maximum functional responses; a high-efficacy agonist needs to occupy fewer receptors to produce a maximal response than a low-efficacy agonist (also known as a partial agonist).

Retrograde synaptic messengers
Compounds that are released by a postsynaptic dendrite or cell body, but that act presynaptically — for example, to influence the release of a transmitter.

Structure–activity relationship
(SAR). The relationship between the pharmacological activity of compounds and their chemical structures.

Transient receptor potential cation channel subfamily V member 1
(TRPV1). A member of a superfamily of transmembrane cation channels; it was previously known as vanilloid receptor 1.

of phospholipase C, which can catalyse the production of DAG and phospholipase A2 (REF. 11) and that it can both inhibit the uptake of adenosine by cultured microglia and macrophages and affect the synaptosomal uptake of 5-hydroxytryptamine (it inhibits this process), of noradrenaline (it enhances this process) and of dopamine (it both enhances and inhibits this process)[85,87]. In addition, at higher concentrations, Δ^9-THC has been found to affect several other such pharmacological targets[85,87]. For example, at concentrations between 1 μM and 10 μM, it has been reported to enhance the activation of β-adrenoceptors, to function as a negative allosteric modulator of μ- and δ-opioid receptors, to activate the cation channels TRPV3 and TRPV4 and to inhibit T-type calcium (Ca_v3) and potassium ($K_v1.2$) voltage-gated ion channels, as well as conductance in Na^+ voltage-gated ion channels. In this concentration range, Δ^9-THC has also been reported to inhibit the enzymes lipoxygenase, Na^+–K^+-ATPase and monoamine oxidase, as well as the cytochrome P450 enzymes CYP1A1, CYP1A2, CYP2B6 and CYP2C9, to inhibit noradrenaline-induced melatonin biosynthesis, and to activate or to inhibit Mg^{2+}-ATPase[85,87].

Perspectives

There has been much progress in our understanding of the plant cannabinoids and of CB1 and CB2. We have identified endogenous lipid mediators that act on these receptors to regulate multiple pathways of cellular signalling. We have discovered synthetic agonists and antagonists for these receptors as well as allosteric modulators of CB1. However, there is still much more knowledge to be gained and challenges to be met in the fields of cannabinoid receptor neuroscience, pharmacology, molecular biology and cannabinoid medicine.

We now need to understand how the endocannabinoid receptors interact with other proteins in complexes that regulate differentiated functions both at the cell surface and in intracellular organelles, particularly in the brain[91–93].

Dozens of endogenous molecules, with structures that resemble those of the endocannabinoids, have been discovered in the brain[94,95]. The activity of most of these molecules is not known. Some of those that have been investigated show activities that have therapeutic potential; for example, arachidonoyl serine is a vasodilator[96] and is neuroprotective after brain injury as it reduces apoptosis[97]. It leads to proliferation of neural progenitor cells *in vitro* and maintains these cells in an undifferentiated state *in vitro* and *in vivo*. Although it does not bind to CB1 and CB2, its activity is blocked by CB2 antagonists[98]. This raises questions, such as what is the relationship of such endocannabinoid-like compounds to the endocannabinoid system and what are the physiological roles of these molecules in the brain?

Pucci *et al.*[99] have investigated the possible epigenetic regulation of skin differentiation genes by phytocannabinoids[99]. CBD was found to increase DNA methylation of the keratin 10 gene. Remarkably, CBD also reduced keratin 10 mRNA levels by a CB1-dependent mechanism. Thus, in this system, CBD is apparently a transcriptional repressor that can control cell proliferation and differentiation. As anandamide has also been found to have epigenetic properties[100], it is of interest to determine the extent, if any, of transcriptional control by endocannabinoids by epigenetic mechanisms.

Although various methods have been used to enhance endocannabinoid levels *in vivo* (even in patients)[82,101], neither anandamide nor 2-AG have been administered to humans. In addition, only a small number of clinical studies have been carried out using plant cannabinoids. A notable exception is the recent successful clinical trial using CBD in schizophrenic patients[101]. Although it is widely mentioned in the general media that cannabis with a high concentration of CBD is therapeutic in paediatric epilepsy and that 'medical marijuana' is indeed of value in such cases[102], there have not been any recent clinical trials reported, although several such trials are ongoing (an anti-epileptic trial of CBD in adults was reported 34 years ago[103]).

In a recent review, Pacher and Kunos[84] suggested that "modulating endocannabinoid system activity may have therapeutic potential in almost all diseases affecting humans". They supported this strong statement with a long list of examples, although these examples were mostly obtained *in vitro* or from *in vivo* experiments in animals[84]. If this summary of effects is shown to reflect actions in human patients, is the endocannabinoid system going to bring a revolution in therapy? This might be the case as investigators are now able to target multiple cell-specific synthetic and biotransformation enzyme pathways that can adjust the levels of endocannabinoid ligands with some degree of tissue selectivity. In addition, aside from the agonist and antagonist ligands for cannabinoid receptors, researchers can now target cell type-specific allosteric modulators and receptor-associated proteins. Thus, there is great promise for the future of cannabinoid research.

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Competing interests statement

The authors declare no competing interests.