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Cannabinoid pharmacology: the first 66 years

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Research into the pharmacology of individual cannabinoids that began in the 1940s, several decades after the presence of a cannabinoid was first detected in cannabis, is concisely reviewed. Also described is how this pharmacological research led to the discovery of cannabinoid CB_1 and CB_2 receptors and of endogenous ligands for these receptors, to the development of CB_1- and CB_2-selective agonists and antagonists and to the realization that the endogenous cannabinoid system has significant roles in both health and disease, and that drugs which mimic, augment or block the actions of endogenously released cannabinoids must have important therapeutic applications. Some goals for future research are identified.
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Abbreviations: CBD, cannabidiol; CBN, cannabinol; MPLM, myenteric plexus longitudinal muscle; THC, tetrahydrocannabinol; THCV, tetrahydrocannabivarin

The discovery of cannabinoids

Cannabis is one of the first plants to have been used as a medicine, for religious ceremonies and recreationally, the first accounts of its use for these purposes stretching back 5000 years (reviewed in Mechoulam, 1986). However, the findings that cannabis is the unique source of a set of at least 66 compounds now known as cannabinoids (Table 1 and ElSohly, 2002) and that the psychotropic effects of cannabis are produced mainly by (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC; Figure 1) are much more recent.

Cannabinol (CBN; Figure 1), much of which is thought to be formed from THC during the storage of harvested cannabis, was the first of the plant cannabinoids (phytocannabinoids) to be isolated, from a red oil extract of cannabis, at the end of the 19th century. Its structure was elucidated in the early 1930s by R.S. Cahn, and its chemical synthesis first achieved in 1940 in the laboratories of R. Adams in the U.S.A. and Lord Todd in the U.K. A second phytocannabinoid, (−)-cannabidiol (CBD; Figure 1), was first obtained from cannabis in the same year by Adams and colleagues, probably in combination with cannabidiolic acid, while THCs were first extracted from cannabis in 1942 by Wollner, Matchett, Levine and Loewe, most likely as a mixture of (−)-Δ^8- and (−)-Δ^9-THC (Figure 1). Both THC and CBD are present in cannabis mainly as acids that are decarboxylated when cannabis is heated. The structures and stereochemistry of CBD and Δ^9-THC, each of which occurs naturally as its (−)-enantiomer, were elucidated in Raphael Mechoulam's laboratory: in 1963 for CBD and in 1964 for Δ^9-THC, when it was first isolated from cannabis. It was also in Mechoulam's laboratory, in 1965, that (±)-Δ^9-THC and (±)-CBD were first synthesized, developments that were soon followed by the synthesis of the (+)- and (−)-enantiomers, both of these two cannabinoids and of Δ^8-THC. These important advances and the identification of many of the other cannabinoids present in cannabis are described in greater detail elsewhere (Mechoulam, 1973; Mechoulam & Hanus, 2000).

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Early research into the pharmacology of cannabinoids

Pharmacological experiments with single cannabinoids were first performed in the 1940s and 1950s (reviewed in Loewe, 1944; Paton & Pertwee, 1973a). Many of these were carried out either with preparations of THC, CBN or CBD extracted from cannabis or with two then recently synthesized cannabinoids, $\Delta^{6a,10a}$-THC (Figure 1) (reviewed in Mechoulam, 1973; Mechoulam & Hanus, 2000), and its hexyl analogue, synhexyl (pyrahexyl, parahexyl; Figure 1) (Loewe, 1946), neither of which are present in cannabis. Among the first pharmacological observations to have been made with individual cannabinoids are those of Loewe (1946), who noted that THC and synhexyl, but not CBD, induced catalepsy in mice, that CBN induced catalepsy in mice but only at high doses that were also lethal, that THC and synhexyl had a central excitant action, particularly in rabbits and mice, and that THC and synhexyl but not CBN or CBD elicited corneal areflexia in rabbits. These were, of course, some of the first indications that cannabinoids exhibit marked structure–activity relationships. Since it is now generally accepted that the ability of cannabinoids to produce signs of catalepsy in rodents correlates well with their psychotropic activity (see below), these results also provided early evidence that CBN has much lower potency than THC as a psychotropic agent, and that CBD lacks psychotropic activity altogether. An even earlier experiment, carried out by Haagen-Smit *et al.* (1940), showed that a purified extract of cannabis ('cannin'), that must have

Table 1 Plant-derived cannabinoids (phytocannabinoids)

- Δ^9-tetrahydrocannabinol-type (9)
- Δ^8-tetrahydrocannabinol-type (2)
- Cannabidiol-type (7)
- Cannabigerol-type (6)
- Cannabichromene-type (5)
- Cannabicyclol-type (3)
- Cannabielsoin-type (5)
- Cannabitriol-type (9)
- Miscellaneous-type (11)
- Cannabinol and cannabinodiol-types (air-oxidation artefacts)

The number of each of the listed types of cannabinoid that has been found in cannabis is shown in parenthesis (reviewed in ElSohly, 2002). Tincture of cannabis (right hand panel) was a commercial product that was prepared from *Cannabis sativa* grown in Pakistan and imported into Britain under licence (Gill *et al*., 1970).

approximated closely to Δ^9-THC, shared the ability of a crude cannabis preparation to produce seemingly aimless scratching behaviour and signs of motor incoordination and catalepsy in a dog.

Another finding made at this time, that sleep induced in mice by an unnamed barbiturate can be prolonged by CBD, although not by higher doses of CBN or THC, is also attributable to Loewe (1944). This observation led to the finding that CBD is much more active than Δ^9-THC as an inhibitor of the hepatic metabolism of phenazone (Paton & Pertwee, 1972), and that this inhibition depends on the ability of CBD or a CBD metabolite to inhibit certain microsomal cytochrome P450 (CYP) enzymes (reviewed in Pertwee, 2004). There is now also evidence that CBD can induce hepatic CYP3A, CYP2B and CYP2C, and that the structure–activity relationships of CBD analogues for CYP inhibition and CYP induction are not the same (Pertwee, 2004). It is noteworthy that the older literature refers to Δ^9-THC, Δ^8-THC and $\Delta^{6a,10a}$-THC (formal pyran numbering system) as Δ^1-THC, Δ^6-THC and Δ^3-THC, respectively (monoterpenoid numbering system). While formal pyran numbering is now most commonly used for THC, only the monoterpenoid numbering system is valid for CBD, so that the carbon atoms of CBD and THC are numbered differently (Figure 1).

In the mid-1960s and early 1970s research into the pharmacology of cannabinoids increased markedly. This was mainly in response to the widespread use of cannabis as a recreational drug in the U.K. and other Western countries, and was facilitated by the structural elucidation and synthesis of Δ^9-THC at that time. In contrast, there was less interest in the therapeutic potential of cannabinoids even though tincture of cannabis was then still a licensed medicine in the U.K. (Table 1). Consequently, cannabinoid experiments focused mainly on the psychoactive properties of cannabis, one important objective being to test the hypothesis that its psychotropic properties were largely attributable to Δ^9-THC (reviewed in Paton & Pertwee, 1973b). This was achieved by comparing various effects of cannabis and Δ^9-THC, not only in animal experiments but also in human studies, which, for example, exploited the ability of cannabis to elevate mood or cause dysphoria, to precipitate psychopathological symptoms such as feelings of anxiety, panic or paranoia, to cause 'felt time' to pass more slowly than 'clock time', to produce changes in auditory and visual perception, to impair memory and to induce drowsiness. The results obtained indicated that the psychotropic effects of cannabis could indeed be attributed essentially just to Δ^9-THC.

Pharmacological research at this time was also directed at seeking out and characterizing the effects of cannabis or individual cannabinoids on particular biological systems, at comparing the effects of cannabis with those of other recreational drugs and at exploring the dependence liability of cannabis and Δ^9-THC. This early research provided a more complete description of the pharmacological effects of cannabis and Δ^9-THC, but did little to explain the mechanisms by which these effects were produced.

In the 1970s, a time when the CYP system of drug-metabolizing enzymes was attracting a great deal of attention, considerable effort was also devoted to characterizing the pharmacokinetics and metabolic fate of Δ^9-THC and other plant cannabinoids in a number of species including man (reviewed in Agurell *et al*., 1986). Largely as a result of the research carried out at that time, it is now generally accepted that for the metabolism and elimination of Δ^9-THC, there is an initial hydroxylation by hepatic CYP enzymes to its main primary metabolite, 11-hydroxy-Δ^9-THC, which retains Δ^9-THC-like pharmacological activity, and to several other hydroxylated compounds, some of which also exhibit such activity. The phase I metabolites of Δ^9-THC are converted in the liver to glucuronides and, after their biliary excretion into the intestinal tract, these glucuronides undergo enzymic hydrolysis to 11-hydroxy-Δ^9-THC and Δ^9-THC-11-oic acid.

An important prerequisite for seeking out the modes of action of any drug is the availability of quantitative bioassays. For the cannabinoids, two bioassays that proved to be successful measured 'static ataxia' in dogs and changes such as sedation, ptosis and body sag in monkeys (reviewed in Howlett *et al*., 2002). These bioassays yielded data that

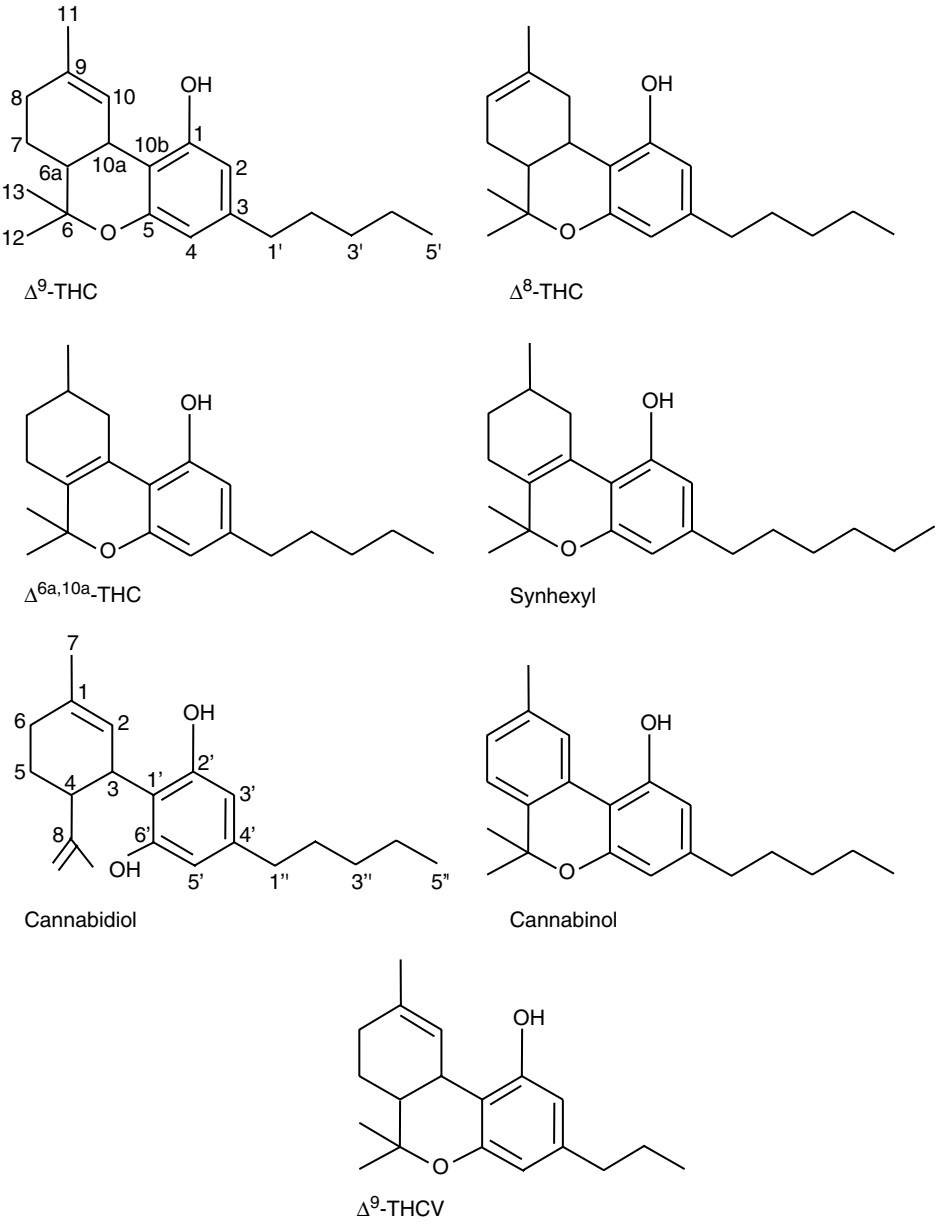

Figure 1 The structures of five plant cannabinoids (phytocannabinoids), Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-THC, cannabidiol (CBD), cannabinol (CBN) and Δ^9-tetrahydrocannabivarin (Δ^9-THCV), and of two synthetic cannabinoids, $\Delta^{6a,10a}$-THC and synhexyl.

supported the hypothesis that Δ^9-THC is the main psychotropic constituent of cannabis. The possibility of using rats or mice instead of dogs or monkeys was also explored and this approach led to the development of several new *in vivo* bioassays. These included four tests that were later combined to form what came to be known as the 'mouse tetrad' (see below). In one of these bioassays, the ring test, mice are placed across an elevated horizontal ring and the proportion of time they remain immobile/cataleptic (immobility index) is monitored over a 5-min period (Pertwee, 1972). Cannabis and psychoactive cannabinoids such as Δ^9-THC cause the immobility index to increase in a dose-related manner. This bioassay was based on an observation by Loewe (1946) that THC extracted from cannabis resin induced a cataleptic state in the mouse that is 'best manifested when the animal is placed prone upon an arrangement (brim of a beaker or two parallel wires) for supporting it only at the thigh and jaws'. Eventually, *in vitro* assays for cannabinoids were also developed and it was two of these in particular, a bioassay that measures adenylate cyclase activity and a radioligand binding assay, that provided conclusive evidence for the existence of the cannabinoid CB_1 receptor.

The discovery of cannabinoid receptors

Early indications of the existence of cannabinoid receptors came from reports firstly, that the pharmacological activity of psychotropic cannabinoids is significantly influenced by chemical structure, secondly, that cannabinoids with chiral

centres exhibit stereoselectivity, and thirdly, that the potency of Δ^9-THC matches that of agonists for at least some established classes of receptor (reviewed in Howlett *et al*., 2002; Pertwee, 2005b).

As detailed elsewhere (Pertwee, 1988), this evidence for the existence of cannabinoid receptors was weakened by findings that psychoactive cannabinoids can produce changes in the physical properties of artificial membranes containing only cholesterol and phospholipid, that there is a correlation between the ability of certain cannabinoids to produce these changes and their psychoactive potency, and that (−)-Δ^9-THC interacts more potently with artificial membranes than its nonpsychotropic enantiomer, (+)-Δ^9-THC. Indeed, it was findings such as these that led Lawrence & Gill to propose in 1975 that 'it is unnecessary to invoke the existence of a specific cannabinoid receptor' and to propose that the psychoactivity of cannabinoids results from a structure-dependent ability to disorder membrane lipids, and that this ability relies on 'awkwardness of fit' into asymmetric components of the hydrocarbon matrix rather than on 'goodness of fit' into a specific receptor (see Pertwee, 1988).

In the mid-1980s, two groundbreaking findings were made in Allyn Howlett's laboratory at St Louis University that provided conclusive evidence that cannabinoid receptors do indeed exist (reviewed in Howlett, 2005). The first of these findings owed much to advances that were taking place at that time in our understanding of signalling by G-protein-coupled receptors and was facilitated by the development by Pfizer of several novel potent cannabinoids. This crucial finding was that psychotropic cannabinoids have in common an ability to inhibit adenylate cyclase by acting through $G_{i/o}$ proteins.

The second major advance in Allyn Howlett's laboratory was made in collaboration with Bill Devane in 1988 (reviewed in Howlett, 2005). This was made possible firstly, by the availability of a then relatively new technique that allowed the presence of the recognition sites of receptors to be detected using a radiolabelled ligand, and secondly, by labelling the Pfizer cannabinoid, CP55940, with tritium. This radioligand proved to be much more suitable than [^{3}H]-Δ^9-THC as a probe for cannabinoid receptors: it has much higher affinity for these receptors and so undergoes less nonspecific binding at concentrations that undergo specific binding. The results obtained with [^{3}H]-CP55940 provided evidence for the presence of high-affinity binding sites for this ligand in rat brain membranes. Since the ability of unlabelled cannabinoids to displace [^{3}H]-CP55940 from these sites and to induce $G_{i/o}$-mediated inhibition of adenylate cyclase was found to correlate with their ability to elicit cannabimimetic responses *in vivo* in mice, it was now almost certain that cannabinoids acted on a receptor and that this receptor was G-protein coupled. Confirmation came with the cloning in 1990 of the rat CB_1 receptor in Tom Bonner's laboratory at NIH and of the human CB_1 receptor by Gérard and colleagues in Brussels and, less expectedly, with the cloning in 1993 of a second G-protein-coupled cannabinoid receptor (CB_2) in Sean Munro's laboratory in Cambridge (reviewed in Howlett *et al*., 2002).

Since the discovery of CB_1 and CB_2 receptors, a great deal has become known about how these receptors signal and about their roles (reviewed in Howlett *et al*., 2002; Howlett, 2005; Pertwee, 2005b). Thus, it is now generally accepted that CB_1 and CB_2 receptors are both coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinase. Additionally, CB_1 receptors are coupled through $G_{i/o}$ proteins to certain ion channels and can also act through G_s proteins, for example, to activate adenylate cyclase. CB_1 receptors are found predominantly but not exclusively at central and peripheral nerve terminals where they mediate inhibition of transmitter release (reviewed in Pertwee, 1997; Howlett *et al*., 2002). Their distribution pattern within the central nervous system accounts for several characteristic effects of CB_1 receptor agonists, including their ability to produce hypokinesia and catalepsy and to induce signs of analgesia in both animals and man (reviewed in Howlett *et al*., 2002; Walker & Hohmann, 2005). CB_2 receptors occur mainly on immune cells, likely roles of these receptors including modulation of cytokine release and of immune cell migration. Although often regarded as peripheral receptors, CB_2 receptors have been detected in the central nervous system, for example, on microglial cells (reviewed in Howlett *et al*., 2002; Pertwee, 2005b).

The discovery of cannabinoid receptors prompted the development of a number of *in vitro* bioassays that could be used to monitor the activation or blockade of these receptors (reviewed in Pertwee, 1997, 2005b; Howlett *et al*., 2002). These bioassays can be performed with cultured cells that have been transfected with CB_1 or CB_2 receptors or with cells or tissues that express CB_1 and/or CB_2 receptors naturally. Some of the most widely used of these bioassays exploit what is currently known about cannabinoid receptor signalling, for example, by monitoring the ability of cannabinoid receptor agonists to stimulate [^{35}S]-GTP-γS binding to G-proteins, to alter the activity of G-protein-coupled intracellular enzymes such as adenylate cyclase or mitogen-activated protein kinase or to modulate intracellular levels of calcium. Others, performed with isolated nerve-smooth muscle preparations such as the mouse vas deferens and the myenteric plexus longitudinal muscle (MPLM) preparation of guinea-pig small intestine, exploit the ability of neuronal CB_1 receptors to mediate a concentration-related inhibition of the electrically evoked release of contractile transmitters, the measured response being the decrease in smooth muscle contractions that results from this inhibition of transmitter release. The guinea-pig MPLM preparation was first used as a bioassay for cannabinoids by Bill Paton in the late 1960s (Gill *et al*., 1970). However, the mouse isolated vas deferens was not used for this purpose until the 1990s, initially in the U.S.A., and here in Aberdeen (reviewed in Pertwee, 1997) where the discovery that this tissue provides a sensitive and quantitative bioassay for CB_1 receptor ligands arose from a collaboration with Alistair Corbett who was using it as a standard bioassay for both synthetic and endogenous opioids in the laboratory of Hans Kosterlitz.

One obvious need at this time was for strategies that could be used to establish whether or not a particular effect of a cannabinoid was cannabinoid receptor-mediated. This need was eventually met, firstly by the development of selective CB_1 and CB_2 receptor antagonists (see below), and later by the breeding of transgenic receptor-deficient mice, $CB_1^{-/-}$, $CB_2^{-/-}$ and $CB_1^{-/-}/CB_2^{-/-}$. However, in the early 1990s when neither selective antagonists nor transgenic mice were available, other strategies were devised (reviewed in Howlett *et al*., 2002; Pertwee, 2005b). For the *in vivo* bioassay of cannabinoids, one of these was to exploit the apparent ability of animals to discriminate between the subjective effects of psychotropic

cannabinoids and those of noncannabinoids or of cannabinoids that lack psychotropic activity. Another *in vivo* strategy, devised in Billy Martin's laboratory at Virginia Commonwealth University, was to compare the ability of a test compound to produce four effects in a group of mice: hypokinesia, hypothermia, catalepsy in the ring test (see above) and antinociception in the tail-flick or hot plate test. One or other of these effects can be produced by a wide range of noncannabinoids. However, in contrast to established CB_1 receptor agonists, many (although not all) noncannabinoids lack activity in at least one of the four tests that form part of this 'mouse tetrad bioassay'. Consequently, at least some degree of selectivity can be achieved by subjecting animals to all four tests. One of the first *in vitro* strategies used to distinguish cannabinoid receptor agonists from other ligands was to perform bioassays either with cells that had been transfected with CB_1 or CB_2 receptors or with membranes obtained from these cells. Another early strategy was applied to the mouse isolated vas deferens and exploited the ability of Δ^9-THC to reduce the sensitivity of this tissue to cannabinoid receptor agonists in a selective manner when it was administered to mice *in vivo* (reviewed in Pertwee, 1997). For validating a particular bioassay, it also proved helpful to establish whether a correlation existed between the potencies exhibited by a set of cannabinoids or by a pair of enantiomeric cannabinoids for displacing a radioligand from CB_1 binding sites and the pharmacological potencies shown by the same compounds in the bioassay under investigation.

The discovery of endogenous cannabinoids

Once cannabinoid receptors had been discovered, it became important to establish whether mammalian tissues also produce a cannabinoid receptor agonist or whether these receptors are targets only for plant cannabinoids and their synthetic cousins. The search for an endogenous cannabinoid had begun. One likely candidate was isolated from pig brain by Bill Devane, who was now working in Jerusalem with Raphael Mechoulam (Devane *et al*., 1992). This was a lipophilic molecule that readily displaced the potent cannabinoid receptor ligand, [^{3}H]-HU243, from rat brain membranes with a K_i value of 52 nM. To establish whether this endogenous ligand would activate CB_1 receptors, a few micrograms were sent to Aberdeen where it was found that this test material did indeed share the ability of CB_1 receptor agonists to inhibit electrically evoked contractions of the mouse isolated vas deferens (Devane *et al*., 1992). Moreover, it produced this inhibitory effect in a naloxone-insensitive manner and with an EC_{50} value that approximated to its CB_1 K_i value, a finding that is in line with its subsequent classification as a CB_1 receptor partial agonist (reviewed in Howlett *et al*., 2002). The material was then synthesized, identified as arachidonoyl ethanolamide (Figure 2), and named anandamide from 'ananda', the Sanskrit word for 'bliss'. Evidence that anandamide was acting through CB_1 receptors in the vas deferens was initially obtained by demonstrating that tissues rendered tolerant to established CB_1 receptor agonists but not to noncannabinoid inhibitors of electrically evoked contractions such as clonidine or opioid receptor agonists (reviewed in Pertwee, 1997) also exhibit tolerance to anandamide (Pertwee *et al*., 1993). It was subsequently confirmed that anandamide is

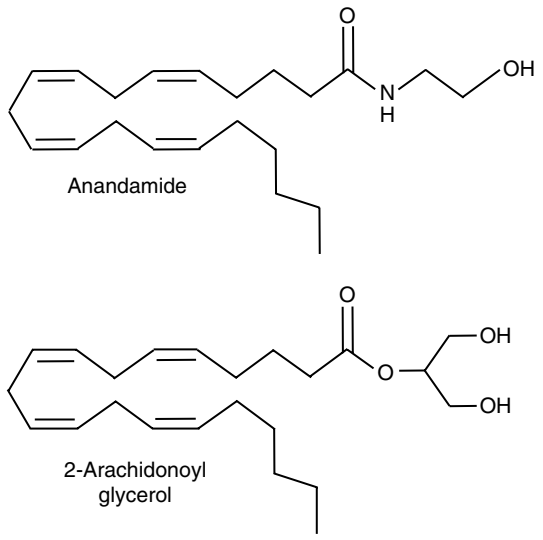

Figure 2 The structures of two endocannabinoids, anandamide and 2-arachidonoyl glycerol.

active in other established bioassays for cannabinoid receptor agonists (reviewed in Pertwee, 1997; 1999) and, once the first CB_1-selective antagonist, SR141716A, had been developed (see below), that anandamide is susceptible to antagonism by this ligand (Rinaldi-Carmona *et al*., 1994). It was fortuitous that the first isolated tissue experiments with the minute amounts of anandamide that had been extracted from pig brain were not carried out with the guinea-pig MPLM preparation, then also being used in Aberdeen for the bioassay of cannabinoids, as it subsequently became apparent that anandamide is rapidly metabolized by this guinea-pig preparation but not by the mouse vas deferens (Pertwee *et al*., 1995).

The discovery of anandamide was followed by reports that mammalian tissues contain a number of other fatty acid derivatives that behave as endogenous cannabinoids (reviewed in Di Marzo *et al*., 2005; Pertwee, 2005c). Apart from anandamide, the most investigated of these has been 2-arachidonoyl glycerol (Figure 2). There is evidence that both these endogenous cannabinoids are synthesized on demand rather than stored, and that following their release, they are removed from their sites of action by cellular uptake processes that for anandamide probably involve a combination of simple diffusion and facilitated, carrier-mediated transport (reviewed in Hillard & Jarrahian, 2003). They are then metabolized intracellularly, anandamide by fatty acid amide hydrolase and 2-arachidonoyl glycerol mainly by monoacylglycerol lipase (reviewed in Di Marzo *et al*., 2005). Most of the endogenous cannabinoids that have so far been identified are high- or low-efficacy cannabinoid receptor agonists. However, one of them, virodhamine, has been found in some experiments to behave as a CB_1 receptor antagonist/inverse agonist (reviewed in Pertwee, 2005c).

The development and pharmacological characterization of cannabinoid receptor ligands

At the time of the discovery of the cannabinoid CB_1 receptor, there were just two main chemical classes of psychotropic

cannabinoids, the 'classical cannabinoids' that consist of tricyclic dibenzopyrans such as Δ^9-THC and its far more potent synthetic analogue (−)-11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210), and the 'nonclassical' cannabinoids of which the bicyclic CP55940 and tricyclic CP55244 are important members. Subsequently, other chemical classes of psychotropic cannabinoids made their appearance, for example, the aminoalkylindole *R*-(+)-WIN55212, endogenous eicosanoids such as anandamide and 2-arachidonoyl glycerol (see above) and, more recently, the Bayer compound, BAY 38-7271 (reviewed in Howlett *et al*., 2002; Pertwee, 2005b). All these compounds proved to be agonists for both CB_1 and CB_2 receptors that bind more or less equally well to each receptor type but that vary in their CB_1 and CB_2 affinities and relative intrinsic activities. Agonists that activate CB_1 receptors or CB_2 receptors selectively have also been developed.

One other major advance prompted by the discovery of cannabinoid receptors was the development of selective cannabinoid receptor antagonists (reviewed in Howlett *et al*., 2002; Pertwee, 2005b). Among these were the CB_1-selective ligand SR141716A, the development of which was announced in 1994 by Rinaldi-Carmona *et al*., and the CB_2-selective ligand SR144528, which made its first appearance in 1998. Other notable antagonists to be developed in the 1990s were the CB_1-selective LY320135 and three compounds designed and synthesized by Alexandros Makriyannis: the CB_1-selective AM251 and AM281, which are both analogues of SR141716A, and the CB_2-selective aminoalkylindole AM630. More recently, it has been found that cannabis can produce its own cannabinoid receptor antagonist, Δ^9-tetrahydrocannabivarin (Figure 1) (Thomas *et al*., 2005), the presence of which in cannabis was first detected by Edward Gill (Gill *et al*., 1970). The availability of selective CB_1 and CB_2 receptor antagonists (and agonists) has greatly facilitated research into the pharmacology of cannabinoids.

It soon became clear that, when administered by themselves, the 'first generation' of cannabinoid receptor antagonists were capable of producing effects opposite in direction from those produced by CB_1 or CB_2 receptor agonists. Such 'inverse cannabimimetic effects' can result from antagonism of endogenously released cannabinoids. However, some inverse cannabimimetic effects appear to be produced in the absence of any ongoing endogenous cannabinoid release, prompting the hypothesis that cannabinoid receptors can exist in a constitutively active state in which they undergo some degree of coupling to their effector mechanisms, even in the absence of an agonist and that inverse effects at these receptors can be induced by a process of 'inverse agonism' in which these receptors are shifted from a proposed constitutively active 'on' state to one or more constitutively inactive 'off' states (reviewed in Pertwee, 2005a). One recent advance that is consistent with this hypothesis has been the development of 'neutral' competitive CB_1 receptor antagonists. These antagonists seem to lack the apparent ability of ligands such as SR141716A to reduce the degree of any constitutive activity exhibited by CB_1 receptors (reviewed in Pertwee, 2005a).

A common property of all cannabinoid receptor agonists and antagonists currently used as experimental tools is one of high lipophilicity and low or negligible water solubility. This necessitates the use of a vehicle such as dimethyl sulphoxide, Tween-80 or ethanol, which may itself produce pharmacological changes or influence the free concentration of a cannabinoid at its site of action. This practical difficulty prompted an exploration of the possibility of developing a water-soluble cannabinoid receptor agonist, leading to the synthesis by Raj Razdan of O-1057, a classical cannabinoid that is readily soluble in water and yet almost as potent as CP55940 as a CB_1 and CB_2 receptor agonist (Pertwee *et al*., 2000).

It is now generally accepted that, in contrast to 2-arachidonoyl glycerol and established non-eicosanoid cannabinoids, anandamide can activate not only CB_1 and CB_2 receptors but also vanilloid TRPV1 receptors (reviewed in Ross, 2003). In addition, evidence has recently emerged that the orphan G-protein-coupled receptor, GPR55, is a cannabinoid receptor (see, e.g. Brown & Wise, 2003), and there is also evidence for several other pharmacological targets for cannabinoids (reviewed in Pertwee, 2005b). As cannabinoid receptor agonists do not interact with each of these proposed additional targets to the same extent, it follows that they are likely to possess different pharmacological profiles in spite of their shared ability to activate CB_1 and/or CB_2 receptors. This should be borne in mind when selecting a cannabinoid receptor agonist for use as a pharmacological tool or potential medicine. Also, the possibility still remains that cannabinoids produce some of their effects by inducing structure-dependent perturbations of membrane lipids as proposed by Edward Gill and David Lawrence (see above). One other recent finding of note is that the CB_1 receptor has an allosteric site (Price *et al*., 2005), opening up the possibility of developing non-cannabinoids that modify responses to endogenously released cannabinoids through allosteric modulation of the receptor.

Tolerance and dependence

Results from experiments conducted during the 1970s indicated that tolerance can develop to many of the effects of cannabis and Δ^9-THC, that this is induced more readily and rapidly to some effects than to others and that it is essentially pharmacodynamic in nature and does not depend to any significant extent on changes in cannabinoid disposition or metabolism (reviewed in Pertwee, 1991; Sim-Selley, 2003; Tanda & Goldberg, 2003). When psychoactive cannabinoids other than Δ^9-THC were developed, it became clear that these too can induce tolerance. However, a fuller elucidation of the mechanisms that underlie the development of this tolerance had to await the discovery of cannabinoid receptors. It then became possible to establish, at least for effects mediated by cannabinoid CB_1 receptors, that internalization of these receptors with or without their subsequent degradation, decreases in CB_1 receptor protein synthesis, and reductions in the efficiency of CB_1 receptor signalling (desensitization) can all contribute to the development of tolerance to agonists for these receptors. Interestingly, the extent to which any one of these mechanisms is involved in the production of this tolerance seems to be brain area-dependent and also to be influenced by agonist efficacy. Not much is presently known about tolerance to effects mediated by cannabinoid CB_2 receptors.

It has also long been known that repeated administration of cannabis or Δ^9-THC can give rise to a 'physical' abstinence

syndrome when either of these is abruptly withdrawn from humans or animals (reviewed in Pertwee, 1991). This syndrome is not particularly pronounced, probably because Δ^9-THC is highly lipophilic and so disappears only very slowly from its sites of action. However, following the development of SR141716A, it became possible to show that animals repeatedly pretreated with a cannabinoid receptor agonist and then challenged with this CB_1 receptor antagonist can exhibit quite an intense abstinence syndrome (reviewed in Tanda & Goldberg, 2003). With regard to the possibility that CB_1 receptor agonists such as Δ^9-THC or *R*-(+)-WIN55212 have a rewarding effect, it is only quite recently that this has been demonstrated unequivocally in self-administration experiments with animals (reviewed in Tanda & Goldberg, 2003). Other indications that CB_1 receptor agonists have a rewarding effect have come from animal experiments in which Δ^9-THC was shown to lower the reward threshold of certain strains of rat for intracranial self-stimulation or in which the conditioned place preference procedure was used. It is likely that Δ^9-THC can produce both rewarding and aversive effects in animals as it has been reported to induce conditioned place preference in some rat or mouse experiments but conditioned place aversion in others (reviewed in Tanda & Goldberg, 2003).

The endocannabinoid system in health and disease

Endogenous cannabinoids are now generally referred to as 'endocannabinoids' and, together with cannabinoid receptors, constitute the 'endocannabinoid system'. The discovery of this system has had a major impact on cannabinoid research which now focuses not only on the pharmacology of phytocannabinoids and their synthetic analogues but also on the pharmacology of the endocannabinoids, on the physiological and pathological events that trigger their release and subsequent cellular uptake and metabolism, and on the roles that endocannabinoids and their pharmacological targets play in both health and disease. As a result, there is, for example, already evidence that one or more of the endocannabinoids serve as retrograde messengers at central synapses (reviewed in Vaughan & Christie, 2005).

Evidence has also emerged that tissue concentrations of endocannabinoids, cannabinoid receptor density and/or cannabinoid receptor coupling efficiency increase in a range of disorders (reviewed in Pertwee, 2005c). In some of these disorders, for example, multiple sclerosis, certain types of pain, cancer, schizophrenia, post-traumatic stress disorders, some intestinal and cardiovascular diseases, excitotoxicity and traumatic head injury, this upregulation of the endocannabinoid system may cause a reduction in the severity of symptoms or a slowing of disease progression. However, there are other disorders, for example, impaired fertility in women, obesity, cerebral injury in stroke, endotoxaemic shock, cystitis, ileitis and paralytic ileus, in which the unwanted effects appear to result from an upregulation of the endocannabinoid system, suggesting that this system has its own pathology and possibly also that it sometimes mediates unwanted effects because it is being influenced by pathological events taking place in some other system from which it receives input. This evidence has prompted a search for the best clinical strategies that will, on the one hand, mimic or augment endocannabinoid-mediated 'autoprotection' and, on the other hand, prevent endocannabinoid-mediated 'autoimpairment' (see below).

Clinical strategies

Research into the therapeutic potential of individual cannabinoids began in the 1970s, ironically at a time when tincture of cannabis had just been withdrawn as a medicine in the U.K. because it was then perceived as having no advantages over more recently developed non-cannabinoid medicines and because a major concern of the regulatory authorities at that time was the widespread recreational use of cannabis. In response to an ever-growing number of reports that cannabis and Δ^9-THC suppress signs of pain in various experimental models, Pfizer began to develop synthetic analogues of THC as potential analgesics. Although this research programme was never completed, it did generate an important set of novel cannabinoid receptor agonists that played a major role in the discovery of the CB_1 receptor (see above). There was also interest in the appetite-stimulating and antiemetic properties of Δ^9-THC and these effects did come to be exploited in the clinic by the 1980s when Δ^9-THC (dronabinol, Marinol®) and its synthetic analogue, nabilone (Cesamet®), both became licensed as medicines for suppressing nausea and vomiting produced by chemotherapy (both drugs) or for stimulating appetite in AIDS patients (dronabinol) (reviewed in Robson, 2005).

More recently, attention has again focused on the possibility of using cannabinoids as analgesics. Indeed, Sativex®, a cannabis-based medicine that contains both Δ^9-THC and CBD (reviewed in Robson, 2005), was recently licensed in Canada as adjunctive treatment for the symptomatic relief of neuropathic pain in adults with multiple sclerosis. Attention is also being directed at other therapeutic applications for cannabinoid receptor agonists that include the relief of other kinds of pain, the management of the spasms and spasticity of multiple sclerosis, and the treatment of intestinal disorders and of certain kinds of cancer. In addition, there is now considerable interest in developing new strategies that might improve the benefit to risk ratio of cannabinoid receptor agonists (reviewed in Pertwee, 2005c). Potential strategies include the administration of

- a CB_2 rather than a CB_1 receptor agonist for pain relief;
- a CB_1 receptor agonist in combination with an opioid at doses that are mutually synergistic, again for pain relief;
- a CB_1 and/or CB_2 receptor agonist that does not readily cross the blood–brain barrier; and
- a CB_1 and/or CB_2 receptor agonist by intrathecal injection or by direct application to some other site outside the brain such as the skin.

It may also prove possible to exploit the 'autoprotective' upregulation of the endocannabinoid system that occurs in some disorders (see above). This might be achieved by treating patients with an inhibitor of endocannabinoid cellular uptake or metabolism, with an allosteric enhancer of the CB_1 receptor (Figure 3) or, for disorders in which there is a 'protective' upregulation of cannabinoid receptor expression level and/or coupling efficiency, by administering a partial cannabinoid

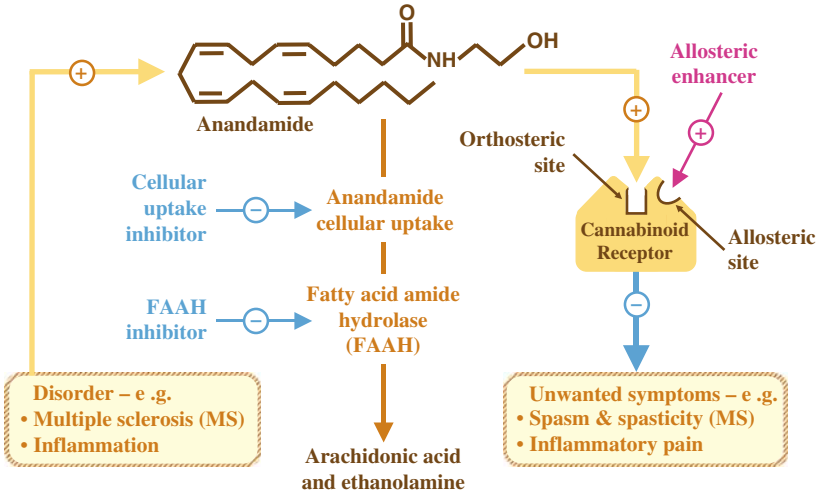

Figure 3 Potential clinical strategies for the management of disorders in which an increased production of anandamide may lead to a reduction in the intensity of unwanted signs and symptoms (reviewed in Pertwee 2005c). These strategies rely on augmentation of apparent anandamide-mediated protective effects through inhibition of the cellular uptake of anandamide, through inhibition of its intracellular metabolism by fatty acid amide hydrolase or through allosteric enhancement of anandamide-induced CB_1 receptor activation.

receptor agonist such as Δ^9-THC rather than a full agonist (reviewed in Pertwee, 2005c).

Since the discovery of CB_1 receptors and the subsequent development of SR141716A by Sanofi in 1994, there has also been considerable interest in the therapeutic potential of competitive CB_1 receptor antagonists for the management of disorders in which the endocannabinoid system appears to induce undesirable symptoms following its upregulation (see above). Indeed, SR141716A (rimonabant) will most likely soon be licensed for use as an antiobesity agent (Van Gaal *et al.*, 2005). Allosteric CB_1 receptor antagonists have potential as medicines too, as do CB_2 receptor inverse agonists since evidence has recently emerged that these can ameliorate inflammation by inhibiting immune cell migration (reviewed in Pertwee, 2005c).

Finally, some pharmacologically active cannabinoids that do not activate or block CB_1 or CB_2 receptors also have therapeutic potential. Among these are the phytocannabinoid, CBD, which, for example, possesses anti-inflammatory, antioxidant and neuroprotective properties (reviewed in Pertwee, 2005b; Robson, 2005).

Future directions

Important milestones in the pharmacohistory of individual cannabinoids have been their discovery at the end of the 19th century, their pharmacological characterization which began in the 1940s, the structural elucidation and synthesis of (−)-Δ^9-THC and (−)-CBD in the 1960s and the discovery of the system of cannabinoid receptors and endogenous ligands for these receptors that is now generally referred to as the endocannabinoid system. These advances owe much to:

- a series of important early contributions to the field that were made by chemists,
- a number of highly productive interdisciplinary collaborations, particularly between medicinal chemists and pharmacologists,
- the development of sensitive *in vivo* and *in vitro* bioassays for cannabinoids,
- the successful design and synthesis of a new generation of potent CB_1 and CB_2 receptor agonists and of potent CB_1 and CB_2 receptor antagonists,
- the emergence of powerful novel techniques that, for example, make it possible for receptors to be labelled with a radioligand, cloned or genetically deleted, or that allow cloned receptors to be transfected into cultured cells, and
- developments in other areas of research, not least receptor signalling.

The challenge now is to continue investigations into the physiological and pathophysiological roles of the endocannabinoid system and to identify and implement the best strategies for exploiting what emerges from this research, in the clinic. Another important objective is to extend current knowledge about the pharmacology, firstly of endocannabinoids, and secondly of cannabinoid receptors and their exogenous agonists, inverse agonists and neutral antagonists when these are administered acutely or chronically. It will also be important to characterize proposed non-CB_1, non-CB_2, non-TRPV1 targets for cannabinoids more completely, to elucidate the pharmacology of cannabinoid receptor allosteric sites more fully, to seek out and explore the pharmacology of any as yet unidentified endocannabinoids or pharmacological targets for cannabinoids, to follow-up early indications that cannabinoid receptors may exist as homodimers or form heterodimers or oligomers with one or more other class of coexpressed receptor (reviewed in Pertwee, 2005b) and to continue the task of exploring the pharmacology of plant cannabinoids.

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