Chapter 6

Known Pharmacological Actions of Delta-9-Tetrahydrocannabinol and of Four Other Chemical Constituents of Cannabis that Activate Cannabinoid Receptors

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6.1 Introduction

Cannabis is the unique natural source of a set of chemicals known as phytocannabinoids (ElSohly et al. 2005), and it is one of these phytocannabinoids, (−)-*trans*-delta-9-tetrahydrocannabinol (Δ^9-THC; Fig. 6.1), that is primarily responsible for producing the well-documented effects on perception, mood, emotion, and cognition that together constitute the psychotropic effects of cannabis (Pertwee 1988). The finding that Δ^9-THC is the main psychoactive constituent of cannabis prompted a search for the pharmacological basis for its psychotropic effects, and this led to three further important discoveries: first, of two new G protein-coupled receptors that were named cannabinoid receptor type 1 (CB_1) and type 2 (CB_2); second, that these receptors can both be activated by Δ^9-THC; and third, that this phytocannabinoid produces many of its effects on brain function by activating the CB_1 receptor (Howlett et al. 2002; Pertwee 1997, 2005).

As discussed in greater detail elsewhere (Howlett et al. 2002; Pertwee 2005), cannabinoid CB_1 receptors are found mainly at the terminals of central and peripheral neurons, where they usually mediate inhibition of ongoing release of a number of different excitatory and inhibitory neurotransmitters. The distribution of these receptors within the central nervous system is such that their activation can affect processes such as cognition and memory, alter the control of motor function, and induce signs of analgesia. CB_1 receptors are also expressed by certain non-neuronal cells, including immune cells. As to cannabinoid CB_2 receptors, these are located predominantly in immune cells and, when activated, they can modulate immune cell migration and cytokine release both outside and within the brain (Cabral and Staab 2005; Howlett et al. 2002; Pertwee et al. 2010). There is also evidence that CB_2 receptors are expressed by some neurons in the brain and elsewhere (Pertwee et al. 2010). However, the role of neuronal CB_2 receptors remains to be established. There is evidence too, first, that CB_1 receptors can signal through both $G_{i/o}$ and G_s proteins, second, that CB_1 receptor agonism can cause $G_{i/o}$ protein-mediated activation of A-type and inwardly rectifying potassium currents, and inhibition of N-type and P/Q-type calcium currents, and third, that either CB_1 or CB_2 receptor agonism can lead to $G_{i/o}$ protein-mediated inhibition of adenylyl cyclase and activation of mitogen-activated protein kinase (Howlett 2005; Howlett et al. 2002; Pertwee 2005). Cannabinoid receptors can be activated not only by exogenously administered compounds, but also by endogenous cannabinoids such as

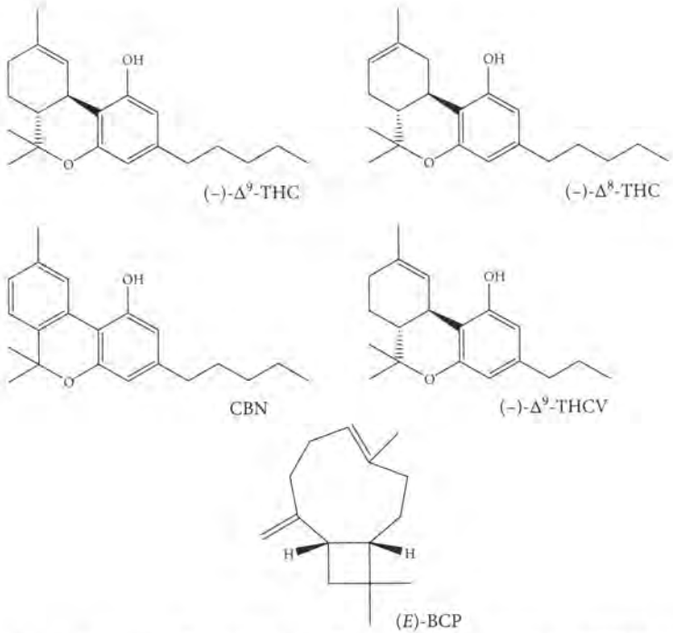

Fig. 6.1 Structures of (–)-*trans*-Δ^9- and (–)-*trans*-Δ^8-tetrahydrocannabinol ((–)-Δ^9-THC and (–)-Δ^8-THC), cannabinol (CBN), (–)-*trans*-Δ^9-tetrahydrocannabivarin ((–)-Δ^9-THCV), and (*E*)-β-caryophyllene ((*E*)-BCP).

N-arachidonoylethanolamine (anandamide) and 2-arachidonoyl glycerol that are synthesized by neurons and other cells and are known as endocannabinoids (Howlett et al. 2002; Pertwee 2005).

This review focuses on Δ^9-THC, and on four other constituents of growing and/or harvested cannabis that have been discovered to activate CB_1 and/or CB_2 receptors: the phytocannabinoids, cannabinol (CBN), (–)-*trans*-delta-8-tetrahydrocannabinol (Δ^8-THC) and (–)-*trans*-delta-9-tetrahydrocannabivarin (Δ^9-THCV), and the sesquiterpene, (*E*)-β-caryophyllene ((*E*)-BCP) (Fig. 6.1). It begins with a brief description of the in vivo and in vitro bioassays that have been used to investigate the ability of these compounds to activate cannabinoid receptors.

6.2 Bioassays for measuring drug-induced activation of cannabinoid receptors in vitro or in vivo

Two in vitro bioassays that are particularly widely used to provide a measure of cannabinoid receptor activation, exploit the ability of CB_1 and CB_2 receptors to signal through $G_{i/o}$ proteins by monitoring the ability of a compound either to increase [^{35}S]GTPγS binding to cell membranes or to inhibit forskolin-induced stimulation of cyclic adenosine monophosphate (AMP) production by whole cells (Howlett et al. 2002; Pertwee 1999; 2005). The strength (efficacy) with which an active compound (agonist) induces receptor activation (agonism) in these assays is usually determined by measuring the size of the maximal response that it can induce (E_{max}), whereas an indication of its potency is usually obtained by establishing the concentration (EC_{50}) at which it produces a half- maximal response.

A third frequently used in vitro bioassay measures the ability of putative cannabinoid receptor agonists to produce complete displacement, from specific binding sites in cannabinoid receptor-expressing membranes, of an established cannabinoid CB_1 and/or CB_2 receptor ligand that has been radiolabeled with tritium (Howlett et al. 2002; Pertwee 1999, 2005). Tritiated cannabinoids often used in such experiments are [^{3}H]CP55940 and [^{3}H]*R*(+)-WIN55212, which bind to both CB_1 and CB_2 receptors, the CB_1-selective ligand, [^{3}H]SR141716A, and the CB_2-selective ligand,

Table 6.1 Examples of K_i values of Δ^9-THC, Δ^8-THC, CBN, Δ^9-THCV, and (*E*)-BCP for the in vitro displacement of [^{3}H]CP55940, [^{3}H]HU-243, or [^{3}H]SR141716A from CB_1- and CB_2-specific binding sites

Displacing compound	CB_1 K_i (nM)	CB_2 K_i (nM)	Reference
Δ^9-THC	5.05	3.13	Iwamura et al. 2001
	8.33[a]	1.73[b]	Iwamura et al. 2001
	13.5[c]	6.8[c]	Iwamura et al. 2001
	21; 40.7[c]	36.4	Showalter et al. 1996
	35.3[c]	3.9[c]	Rinaldi-Carmona et al. 1994
	39.5[d, e]	40[e]	Bayewitch et al. 1996
	47.7[c]	ND	Booker et al. 2009
	53.3	75.3	Felder et al. 1995
	66.5[c,e]; 80.3[d,e]	32.2 [e]	Rhee et al. 1997
Δ^8-THC	44[c]	44	Huffman et al. 1999
	47.6[c]	39.3[a]	Busch-Petersen et al. 1996
CBN	120.2	100	MacLennan et al. 1998
	129.3[c]	ND	Booker et al. 2009
	392.2[c,e]; 211.2[d,e]	126.4[e]	Rhee et al. 1997
	326	96.3	Showalter et al. 1996
	1130	301	Felder et al. 1995
Δ^9-THCV	46.6 [a]	ND	Pertwee et al. 2007
	75.4[a]	62.8	Thomas et al. 2005
	286[c,f]	ND	Hill et al. 2010
	ND	145	Bolognini et al. 2012
	ND	225	Bolognini et al. 2010
(*E*)-BCP	>10,000	155	Gertsch et al. 2008

Abbreviations: CBN, cannabinol;Δ^9-THC, (–)-*trans*-Δ^9-tetrahydrocannabinol; Δ^8-THC, (–)-*trans*-Δ^8-tetrahydrocannabinol; Δ^9-THCV, (–)-*trans*-Δ^9-tetrahydrocannabivarin; (*E*)-BCP, (*E*)-β-caryophyllene; ND, not determined.

Experiments performed with: [a] mouse brain (CB_1) or mouse spleen (CB_2) membranes; [b] membranes from cultured cells transfected with mouse cannabinoid receptors; [c] rat brain (CB_1) or rat spleen (CB_2) membranes; [d] membranes from cultured cells transfected with rat cannabinoid receptors; [e] [^{3}H]HU-243; [f] [^{3}H]SR141716A. All other data are from experiments performed with [^{3}H]CP55940 and/or with membranes from cultured cells transfected with human cannabinoid receptors. See Fig. 6.1 for the structures of the compounds listed in this table.

[^{3}H]SR144528. The potency of an active compound in these binding assays is expressed either as the concentration (IC_{50}) at which it produces 50% displacement of one of these tritiated cannabinoids or as its K_i value (Table 6.1), which can be calculated from its IC_{50} value. K_i values are directly related to the affinities of ligands for their receptors, whereas IC_{50} values are, of course, inversely related to these affinity values.

Moving on to quantitative in vivo bioassays for cannabinoid receptor agonists, these are usually performed with mice or rats, although sometimes with other species, including dogs, pigeons, and nonhuman primates (Howlett et al. 2002). Apparent CB_1 receptor-mediated effects that most often serve as measured responses to drugs in such bioassays are:

- hypolocomotion, hypothermia, antinociception, and catalepsy which, when measured in parallel using mice, constitute the widely used "mouse tetrad" test
- subjective effects which can be distinguished by animals in "drug discrimination" assays from effects produced by substances that do not activate cannabinoid receptors
- impairment of learning and memory as measured, for example, in radial mazes or in the Morris water maze.

Antinociception in the mouse tetrad is most often monitored using tail-flick or hot-plate tests, which provide measures of relief from acute pain induced by heat, whereas catalepsy is often monitored by noting the length of time that mice remain immobile when subjected to a "ring test" that was originally developed in 1972 (Pertwee 1972), or to a "bar test."

As to in vivo indications of CB_2 receptor activation that are exploited in bioassays, these include the reduction of signs of inflammatory paw pain induced in rats or mice by an intraplantar injection of carrageenan or formalin, and the reduction of rat or mouse paw edema induced by intraplantar carrageenan (Bolognini et al. 2010; Guindon and Hohmann 2008).

Importantly, confirmatory evidence that apparent signs of cannabinoid receptor binding or activation observed in CB_1 or CB_2 receptor-transfected cells, or in membranes obtained from these cells, are indeed cannabinoid receptor-mediated can be obtained by establishing whether these signs are, or are not, detectable in untransfected cells. Activation of CB_1 or CB_2 receptors should also be undetectable when an in vitro or in vivo bioassay is performed with animals or tissues from which these receptors have been genetically deleted (Howlett et al. 2002). In addition, a compound that can truly activate CB_1 or CB_2 receptor in an in vivo or in vitro bioassay is expected to be antagonized with appropriate potency by a CB_1-selective antagonist such as SR141716A, AM251, or AM281 and/or by a CB_2-selective antagonist such as SR144528 or AM630 (Howlett et al. 2002; Pertwee 2005).

6.3 Δ^9-tetrahydrocannabinol

6.3.1 Δ^9-THC can activate CB_1 and CB_2 receptors

That Δ^9-THC can activate CB_1 receptors in vivo is strongly supported by the findings, first, that it can, in mice, suppress locomotor activity and induce hypothermia, immobility (catalepsy) in the ring test, and antinociception in the tail-flick test, all at similar doses (Martin et al. 1991), and, second, that its ability to produce each of these tetrad test effects is readily blocked by the selective CB_1 receptor antagonist, SR141716A (Varvel et al. 2005; Wiley et al. 2001). In addition, Δ^9-THC has been found not to affect locomotor activity or to induce hypothermia or ring immobility in mice bred on a C57BL/6J background from which the CB_1 receptor has been genetically deleted (Di Marzo et al. 2000; Zimmer et al. 1999). This genetic deletion also abolished Δ^9-THC-induced antinociception in the hot-plate test, although unexpectedly, not in the tail-flick test.

There is evidence as well that Δ^9-THC can activate CB_2 receptors. Thus, for example, experiments with female mice have shown that Δ^9-THC shares the ability of the CB_2-selective agonist, JWH-133, to decrease the growth rate of xenografts derived from cells that had been isolated from a CB_1- and CB_2-expressing breast cancer tumor, and also, that this effect of Δ^9-THC, and of JWH-133, can be reduced by the CB_2-selective antagonist SR144528 but not by SR141716A (Caffarel et al. 2010). In addition, there has been a report that Δ^9-THC can reduce signs of paw pain in a rat model of arthritis and that this reduction can be attenuated by SR144528 (Cox et al. 2007). This antinociceptive effect of Δ^9-THC was also decreased by SR141716A, suggesting that it was produced through the activation of both CB_1 and CB_2 receptors. It is noteworthy too that, as expected for a CB_2 receptor agonist, Δ^9-THC can decrease carrageenan-induced mouse paw edema (Wise et al. 2008). However, the likely involvement of CB_2 receptors in this effect was not investigated.

Δ^9-THC also behaves as both a CB_1 and a CB_2 receptor agonist in vitro. This is indicated, for example, by its ability to stimulate [^{35}S]GTPγS binding or to inhibit forskolin-induced production of cyclic AMP with significant potency in tissues that express CB_1 receptors, either naturally or after CB_1 receptor transfection (Pertwee 1997; 1999). These effects can be produced by concentrations of Δ^9-THC in the low nanomolar range, although even so, with a potency that is usually less than that displayed by certain other established CB_1/CB_2 receptor agonists such as CP55940 and HU-210 (Pertwee 1997, 1999). Confirmatory evidence that some of these in vitro effects of Δ^9-THC are CB_1 receptor-mediated comes from the finding that they can be prevented by genetic deletion of the CB_1 receptor in the [^{35}S]GTPγS binding assay performed with mouse cerebellar homogenates (Monory et al. 2002), and that cell lines that do not express CB_1 receptors naturally, only exhibit signs of Δ^9-THC-induced CB_1 receptor activation in the cyclic AMP assay if they have first been transfected with this receptor (Matsuda et al. 1990; Slipetz et al. 1995). As expected from the results obtained in these functional in vitro bioassays, it has also been found that Δ^9-THC can fully displace cannabinoid receptor ligands such as [^{3}H]CP55940 from specific binding sites on cannabinoid CB_1 and CB_2 receptors with K_i values in the low nanomolar range (Table 6.1). These K_i values are similar for each of these receptors, but significantly higher than those of the synthetic cannabinoid receptor agonists, CP55940 and HU-210 (Howlett et al. 2002; Pertwee 1997), an indication that Δ^9-THC has less affinity than these other compounds for both CB_1 and CB_2 receptors.

6.3.2 Δ^9-THC is a cannabinoid receptor partial agonist

In several cannabinoid receptor-containing tissue preparations, the maximal sizes (E_{max} values) of apparent CB_1 or CB_2 receptor-mediated effects produced by Δ^9-THC are well below those of certain other established CB_1/CB_2 receptor agonists. This is an indication that Δ^9-THC possesses less CB_1 and CB_2 efficacy than these other agonists and should, therefore, be classified as a partial agonist for these receptors (Pertwee 1997; 1999). Cannabinoids that have been found to display greater CB_1 receptor efficacy than Δ^9-THC, in some in vitro bioassays, include the 11-hydroxy primary metabolite of Δ^9-THC (Matsuda et al. 1990), and the synthetic cannabinoid receptor agonists, CP55940 and HU-210 (Pertwee 1997; 1999). They also include the synthetic cannabinoid, nabilone (Matsuda et al. 1990), which like Δ^9-THC, has been a licensed medicine for many years (Pertwee and Thomas 2009).

Importantly, cannabis is increasingly being taken recreationally together with synthetic "designer drugs" that can activate CB_1 receptors much more strongly than Δ^9-THC (Seely et al. 2012). Just two notable examples of these compounds are JWH-018 and JWH-073, both of which have

been reported to stimulate [^{35}S]GTPγS binding to mouse brain membranes with markedly greater efficacy than Δ^9-THC (Brents et al. 2011; 2012). Pharmacological and toxicological consequences of the recreational use of CB_1 receptor agonists that possess significantly higher efficacy than Δ^9-THC have still to be fully explored.

6.3.3 Δ^9-THC can both activate and block CB_1 and CB_2 receptors

Since Δ^9-THC displays relatively low efficacy as an agonist at CB_1 and CB_2 receptors, it is to be expected that the maximum size of the effect that it can produce when it activates these receptors will be greatly influenced by the proportion of them that are in an "active state" (Bolognini et al. 2012), as well as by their expression level and coupling efficiency, and hence will not be the same in all cannabinoid receptor-expressing tissues. Thus, for example, the size of the maximal effect that Δ^9-THC can produce in tissues in which cannabinoid receptors are particularly highly expressed, or in which they signal with particularly high efficiency, is likely to be quite large. However, in tissues in which cannabinoid receptors are poorly expressed or signal with low efficiency, Δ^9-THC could well fail to produce any detectable sign of cannabinoid receptor activation at all. Indeed, since it would still be expected to possess unchanged affinity for these receptors, Δ^9-THC might possibly antagonize the effects of higher efficacy cannabinoid receptor agonists in such tissues.

It is noteworthy, therefore, that in some in vitro investigations, the maximal sizes of apparent cannabinoid CB_1 receptor-mediated effects of Δ^9-THC have been found to match those of higher efficacy agonists such as CP55940 (Pertwee 1997, 1999), whereas in other investigations, Δ^9-THC has been found to produce signs of antagonism, or even of inverse agonism, at CB_1 or CB_2 receptors either in vitro or in vivo. More specifically, Paronis et al. (2012) have found that in mice, a maximal hypothermic dose of Δ^9-THC (30 mg kg^{-1} s.c.) produced a significant rightward shift in the log dose–response curve of the cannabinoid receptor agonist, AM2389, for its production of hypothermia. By itself, Δ^9-THC behaved as a partial agonist, displaying less hypothermic efficacy than AM2389. There has also been a report that in a mouse model in which CP55940 and *R*-(+)-WIN55212 each produced an apparent anxiolytic effect, Δ^9-THC shared the ability of the CB_1-selective antagonists, SR141716A and AM251, to induce signs of increased anxiety (Patel and Hillard 2006). In addition, in other experiments, Δ^9-THC was found to reduce stimulation of [^{35}S]GTPγS binding to rat cerebellar membranes produced by *R*-(+)-WIN55212 (Sim et al. 1996), to attenuate *R*-(+)-WIN55212- and 2-arachidonoyl glycerol-induced inhibition of glutamatergic synaptic transmission induced in rat or mouse cultured hippocampal neurons (Kelley and Thayer 2004; Shen and Thayer 1999; Straiker and Mackie 2005), or to antagonize CB_2 receptor-mediated inhibition of cyclic AMP production in CB_2-transfected cells (Bayewitch et al. 1996). Moreover, in another investigation, it was found that although Δ^9-THC did, as expected, stimulate [^{35}S]GTPγS binding to membranes obtained from CB_1-transfected cells, it inhibited such binding to membranes obtained from CB_2-transfected cells (Govaerts et al. 2004), an indication that Δ^9-THC can behave as a CB_2 receptor *inverse* agonist. There have also been in vitro investigations in which Δ^9-THC has been found to produce no detectable CB_2 receptor-mediated inhibition of cyclic AMP production (Pertwee 1997, 1999).

6.3.4 CB_1 and CB_2 receptor-independent actions of Δ^9-THC

Among the known CB_1 and CB_2 receptor-independent actions of Δ^9-THC (Table 6.2 and 6.3), are several that it can display at submicromolar concentrations in at least some bioassays and that are, therefore, likely to reduce its CB_1 and CB_2 receptor selectivity. Thus, Δ^9-THC has been reported:

Table 6.2 A selection of receptors and ion channels that Δ^9-THC has been reported to target in vitro

Concentration of Δ^9-THC§	Pharmacological target and effect	Reference
	Receptors and channels	
<1 μM	CB_1 receptor (A or B)	¶
	CB_2 receptor (A or B)	¶
	GPR18 (A)	McHugh et al. 2012
	GPR55 (A)*	Pertwee 2010†
	5-HT_{3A} ligand-gated ion channel (B)	Pertwee 2010†
	Glycine ligand-gated ion channels, including α1 and α1 β1 (P)	Pertwee 2010†
	TRPA1 cation channel (A)*; TRPV2 cation channel (A)*; TRPM8 cation channel (B)	De Petrocellis and Di Marzo 2010†; De Petrocellis et al. 2008, 2011
	PPARγ nuclear receptor (A)	O'Sullivan et al. 2005
	Putative non-CB_1, non-CB_2, non-TRPV1 receptors on capsaicin-sensitive perivascular sensory neurons mediating CGRP release (+)	Zygmunt et al. 2002
1–10 μM	β-adrenoceptor (P)	Pertwee 2010†
	μ-opioid receptors (D)	Pertwee 2010†
	Allosteric modulation of μ- and δ-opioid receptors (–)	Pertwee 2010†
	GPR55 (A or B)	Anavi-Goffer et al. 2012
	PPARγ nuclear receptor (A)	O'Sullivan et al. 2005
	TRPV3 cation channel (A)	De Petrocellis et al. 2012†
	TRPV4 cation channel (A)	De Petrocellis et al. 2012†
	T-type calcium (Ca_v3) voltage gated ion channels (–)	Pertwee 2010†
	Potassium $K_v1.2$ voltage gated ion channels (–)	Pertwee 2010†
	Conductance in Na^+ voltage gated ion channels (–)	Oz 2006†
	Conductance in gap junctions between cells (–)	Oz 2006†
>10 μM	TRPA1 cation channel (A)	De Petrocellis and Di Marzo 2010†
	TRPV2 cation channel (A)	De Petrocellis and Di Marzo 2010†

Abbreviations: 5-HT, 5-hydroxytryptamine; A, activation; B, blockade; CGRP, calcitonin gene-related peptide; D, displacement from binding sites; P, potentiation; PPAR, peroxisome proliferator-activated receptor; TRP transient receptor potential; see also footnote to Table 6.1.

(+), enhancement; (–), inhibition; § EC_{50} or IC_{50} when this has been determined; † review article; * see also effect of 1–10 μM or of >10 μM; ¶ see this review for further details.

Table 6.3 A selection of enzymes and cellular uptake or other processes that Δ^9-THC has been reported to target in vitro

Concentration of Δ^9-THC§	Pharmacological target and effect	Reference
	Enzymes	
<1 μM	Phospholipase(s) (+)*	Pertwee 1988†
	Lysophosphatidylcholine acyl transferase (–)	Greenberg et al. 1978
1–10 μM	Phospholipase(s) (+)	Pertwee 1988†
	Lipoxygenase (–)	Evans 1991
	Na^+-K^+-ATPase activity (–)	Pertwee 1988†
	Mg^{2+}-ATPase activity (±)	Pertwee 1988†
	CYP1A1 (–); CYP1A2 (–); CYP1B1 (–)	Yamaori et al. 2010
	CYP2B6 (–)	Yamaori et al. 2011b
	CYP2C9 (–)	Yamaori et al. 2012
	Norepinephrine-induced melatonin biosynthesis (–)	Koch et al. 2006
	Monoamine oxidase activity (–)	Pertwee 1988†
	Synaptic conversion of tyrosine to noradrenaline and dopamine (+)	Pertwee 1988†
>10 μM	Cyclooxygenase (–)	Evans 1991
	CYP2A6 (–)	Yamaori et al. 2011b
	CYP2D6 (–)	Yamaori et al. 2011c
	CYP3A4 (–); CYP3A5 (–); CYP3A7 (–)	Yamaori et al. 2011a
	Transporters and cellular uptake	
<1 μM	Adenosine uptake by cultured microglia and macrophages (–)	Carrier et al. 2006
	Synaptosomal uptake of dopamine (±)*	Pertwee 1988†
	Synaptosomal uptake of noradrenaline (+)*	Pertwee 1988†
	Synaptosomal uptake of 5-hydroxytryptamine (–)*	Pertwee 1988†
1–10 μM	Cellular uptake of anandamide (–)	Rakhshan et al. 2000
	Synaptosomal uptake of dopamine (–)	Pertwee 1988†
	Synaptosomal uptake of noradrenaline (–)	Pertwee 1988†
	Synaptosomal uptake of 5-hydroxytryptamine (±)	Pertwee 1988†
	Synaptosomal uptake of γ-aminobutyric acid (–)	Pertwee 1988†
	Synaptosomal uptake of choline (–)	Pertwee 1988†
	Other actions or effects	
1–10 μM	Oxidative stress (–)	Marsicano et al. 2002
	Human keratinocyte proliferation (–)	Wilkinson and Williamson 2007
>10 μM	Fluidity of synaptic plasma membranes (+); (–)	Hillard et al. 1985

Abbreviations: (+),enhancement; (–), inhibition; see also footnote to Table 6.1 § EC_{50} or IC_{50} when this has been determined; * see also effect of 1–10 μM or of >10 μM; †review article.

- to inhibit 5-HT_{3A}-mediated currents induced by 5-HT in human embryonic kidney 293 (HEK293) cells stably transfected with the functional 3A subunit of the human 5-HT_3 receptor (IC_{50} = 38 nM), possibly by acting through an allosteric mechanism (Barann et al. 2002)
- to enhance the activation of glycine receptors naturally expressed in rat isolated ventral tegmental area neurons (EC_{50} = 115 nM), and of both homomeric α1 and heteromeric α1β1 subunits of human glycine receptors transfected into *Xenopus laevis* oocytes (EC_{50} = 86 nM and 73 nM, respectively), again possibly in an allosteric manner (Hejazi et al. 2006)
- to elevate calcium levels in HEK293 cells stably overexpressing high levels of the transient receptor potential (TRP) cation channels, TRPA1 or TRPV2 (EC_{50} = 230 nM and 650 nM, respectively), and to desensitize TRPV2 cation channels to activation by lysophosphatidylcholine (IC_{50} = 800 nM) (De Petrocellis et al. 2008, 2011)
- to reduce elevations of intracellular calcium levels induced by the TRPM8 agonists, icilin or menthol, in HEK293 cells stably overexpressing recombinant rat TRPM8 cation channels (IC_{50} = 160 nM and 150 nM, respectively (De Petrocellis et al. 2008; 2011)
- to activate the nuclear receptor, peroxisome proliferator-activated receptor gamma (PPARγ), at concentrations of 100 nM and above in a luciferase reporter gene assay performed with HEK293 cells transiently expressing this receptor (O'Sullivan et al. 2005)
- to activate the G protein-coupled receptor, GPR18 in HEK293 cells transfected with this receptor (EC_{50} = 960 nM; McHugh et al. 2012).

In some in vitro investigations, submicromolar concentrations of Δ^9-THC have also been found to activate GPR55 in HEK293 cells transfected with this deorphanized receptor, both in a β-arrestin assay, albeit with rather low efficacy (Yin et al. 2009), and in a [^{35}S]GTPγS binding assay (EC_{50} = 8 nM; Ryberg et al. 2007). In other in vitro investigations, however, Δ^9-THC induced signs of GPR55 activation only at concentrations in the micromolar range (Anavi-Goffer et al. 2012; Lauckner et al. 2008), or lacked detectable activity as a GPR55 agonist altogether (Pertwee 2010; Pertwee et al. 2010). It is also noteworthy that in one of these investigations (Anavi-Goffer et al. 2012), a concentration of Δ^9-THC (1 μM) that did not seem to activate GPR55, induced a significant downward shift in the log concentration–response curve of an endogenous agonist for this receptor (L-α-lysophosphatidylinositol), when the measured response was stimulation of extracellular receptor kinases 1/2 (ERK1/2) phosphorylation by human GPR55-transfected HEK293 cells.

6.4 Δ^8-tetrahydrocannabinol

Although the pharmacological profile of Δ^8-THC (Fig. 6.1) has been little investigated, there is evidence that it does share the ability of Δ^9-THC to target cannabinoid CB_1 receptors as a partial agonist. Thus, it has been reported to inhibit forskolin-induced production of cyclic AMP in Chinese hamster ovary (CHO) cells transfected with CB_1 receptors with a potency slightly less than that of Δ^9-THC, but an efficacy similar to that of Δ^9-THC and hence less than that of CP55940 (Gérard et al. 1991; Matsuda et al. 1990). This effect of Δ^8-THC was presumably CB_1 receptor-mediated as it was not observed in untransfected CHO cells. In addition, it has been reported first, that Δ^8-THC can fully displace [^{3}H]CP55940 from specific binding sites on cannabinoid CB_1 receptors with a similar potency to Δ^9-THC (Table 6.1), and second, that it also displays similar potency to Δ^9-THC in vivo in the mouse tetrad test (Martin et al. 1993). It has also been found that 11-hydroxy-Δ^8-THC, which is a primary metabolite of Δ^8-THC (Yamamoto et al. 2003), can bind to rat CB_1 and human CB_2 (hCB_2) receptors present in membranes obtained from

African green monkey kidney (COS-7) cells transfected with these receptors, with K_i values in the low nanomolar range (Rhee et al. 1997). Other results obtained in this investigation showed that 11-hydroxy-Δ^8-THC could also inhibit forskolin-induced cyclic AMP production by these cells. Interestingly, however, it displayed much lower CB_2 than CB_1 efficacy as an agonist in the cyclic AMP assay, and yet higher CB_2 than CB_1 affinity in the binding assays.

There is evidence too that Δ^8-THC can induce ataxia in dogs and cannabis-like psychopharmacological effects in human subjects and rhesus monkeys, albeit with less potency than Δ^9-THC (Pertwee 1988). However, whether or not any of these in vivo effects of Δ^8-THC can be opposed by a selective CB_1 receptor antagonist has yet to be investigated. It is noteworthy, therefore, that there have been reports first, that intraperitoneal (i.p.) administration of a low dose of Δ^8-THC can increase food intake by mice, and that this effect can be prevented by the CB_1-selective antagonist, SR141716A (Avraham et al. 2004), and second, that antinociceptive effects induced in a mouse model of acute pain by intracerebroventricular or intrathecal injections of Δ^8-THC, or indeed of Δ^9-THC, can be blocked by this antagonist when it is administered intracerebroventricularly or intraperitoneally (Welch et al. 1998). Finally, again like Δ^9-THC, Δ^8-THC has been found to displace [^{3}H]CP55940 from CB_2 receptors no less potently than it displaces this tritiated ligand from CB_1 receptors (Table 6.1). The likely possibility that Δ^8-THC also activates CB_2 receptors still needs to be investigated, as indeed does the extent to which Δ^8-THC has Δ^9-THC-like CB_1 and CB_2 receptor-independent modes of action.

6.5 Cannabinol

CBN (Fig. 6.1) has been found to bind less potently than Δ^8- or Δ^9-THC to CB_1 and CB_2 receptors, and to possess slightly higher CB_2 than CB_1 affinity (Table 6.1). In addition, it has been found to display lower efficacy than Δ^9-THC as a CB_1 receptor agonist in vitro in both [^{35}S]GTPγS and cyclic AMP assays performed with CB_1-transfected CHO cells, mouse N18TG2 cells, or rat or mouse brain tissue (Pertwee 1999). There has also been one report that CBN activates CB_2 receptors with greater efficacy than Δ^9-THC in the cyclic AMP assay (Rhee et al. 1997), although another report that it behaves as a CB_2 receptor inverse agonist in the [^{35}S]GTPγS binding assay (MacLennan et al. 1998). There is evidence as well that CBN can activate CB_1 receptors in vivo. Thus, it has been found that CBN shares the ability of Δ^9-THC to suppress acetic acid-induced abdominal stretching behavior in mice and that this effect of CBN, like that of Δ^9-THC, can be blocked by SR141716A, but not by SR144528 (Booker et al. 2009). SR141716A has also been reported to prevent increases in food consumption induced in rats by CBN (Farrimond et al. 2012).

Interestingly, 11-hydroxy-CBN seems to target both CB_1 and CB_2 receptors with greater potency than CBN (Table 6.1), since it has been reported to bind to rat CB_1 and hCB_2 receptors with K_i values of 38.0 and 26.6 nM, respectively (Rhee et al. 1997). This 11-hydroxy metabolite of CBN (Yamamoto et al. 2003), has also been found: (1) to activate CB_1 receptors with significant potency (EC_{50} = 58.1 nM), in the cyclic AMP assay performed with rat CB_1-transfected COS-7 cells, but (2) to display little activity as an agonist in this assay (EC_{50} > 10 μM) when it was performed with hCB_2-transfected COS-7 cells, behaving instead as an antagonist of the potent synthetic CB_1/CB_2 receptor agonist, HU-210 (Rhee et al. 1997). Δ^9-THC behaved similarly to 11-hydroxy-CBN in this investigation, displaying significant potency as a CB_1 receptor agonist (EC_{50} = 11 nM) but not as a CB_2 receptor agonist (EC_{50} > 1 μM).

Finally, submicromolar concentrations of CBN have also been found to inhibit CYPA1, CYP1A2, and CYP1B1 enzymes (IC_{50} = 740 nM, 188 nM and 278 nM, respectively), to desensitize

Table 6.4 A selection of receptors, ion channels, enzymes and cellular uptake or other processes that CBN or Δ^9-THCV has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Receptors and channels	
CBN	<1 μM	CB_1 receptor (A)	Pertwee 1999†; Rhee et al. 1997
		CB_2 receptor (A)	Pertwee 1999†; Rhee et al. 1997;
		TRPA1 cation channel (A)*	De Petrocellis et al. 2011
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
	1–10 μM	TRPV1 cation channel (A) (low efficacy)	De Petrocellis et al. 2011
		TRPV3 cation channel (A)	De Petrocellis et al. 2012†
		Conductance in gap junctions between cells (–)	Oz 2006†
		Putative non-CB_1, non-CB_2, non-TRPV1 receptors on capsaicin-sensitive perivascular sensory neurons mediating CGRP release (+)	Zygmunt et al. 2002
	>10 μM	TRPA1 cation channel (A)	De Petrocellis and Di Marzo 2010†
		TRPV2 cation channel (A)	De Petrocellis and Di Marzo 2010†
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
Δ^9-THCV	<1 μM	CB_1 receptor (B)	¶
		CB_2 receptor (A)	¶
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
	1–10 μM	GPR55 (A or B)	Anavi-Goffer et al. 2012
		TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPV1 cation channel (A)	De Petrocellis et al. 2011
		TRPV2 cation channel (A)	De Petrocellis et al. 2011
		TRPV3 cation channel (A)	De Petrocellis et al. 2012†
		TRPV4 cation channel (A)	De Petrocellis et al. 2012†
		Enzymes	
CBN	<1 μM	CYP1A1 (–); CYP1A2 (–); CYP1B1 (–)	Yamaori et al. 2010
	1–10 μM	Phospholipase(s) (+)	Burstein et al. 1982
		Lipoxygenase (–)	Evans 1991
		CYP2B6 (–)	Yamaori et al. 2011b
		CYP2C9 (–)	Yamaori et al. 2012
	>10 μM	Cyclooxygenase (–)	Evans 1991
		CYP3A4 (–); CYP3A5 (–); CYP3A7 (–)	Yamaori et al. 2011a
		CYP2A6 (–)	Yamaori et al. 2011b
		CYP2D6 (–)	Yamaori et al. 2011c

Table 6.4 (continued) A selection of receptors, ion channels, enzymes and cellular uptake or other processes that CBN or Δ^9-THCV has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Norepinephrine-induced melatonin biosynthesis (–)	Koch et al. 2006
		Transporters and cellular uptake	
CBN	1–10 μM	Synaptosomal uptake of dopamine (–)	Poddar and Dewey 1980
		Synaptosomal uptake of noradrenaline (–)*	Poddar and Dewey 1980
	>10 μM	Synaptosomal uptake of noradrenaline (–)	Banerjee et al. 1975
		Synaptosomal uptake of 5-hydroxytryptamine (–)	Banerjee et al. 1975
		Synaptosomal uptake of γ-aminobutyric acid (–)	Banerjee et al. 1975
		Other actions or effects	
CBN	1–10 μM	Oxidative stress (–)	Marsicano et al. 2002
		Human keratinocyte proliferation (–)	Wilkinson and Williamson 2007
	>10 μM	Fluidity of synaptic plasma membranes (+); (–)	Hillard et al. 1985

Abbreviations: A, activation; B, blockade; TRP transient receptor potential; see also footnote to Table 6.1.

(+), enhancement; (–), inhibition; § EC_{50} or IC_{50} when this has been determined; † review article; * see also effect of 1–10 μM or of >10 μM; ¶ see this review for further details.

TRPA1 cation channels to activation by allyl isothiocyanate (IC_{50} = 400 nM) and, like Δ^9-THC, to activate TRPA1 (EC_{50} = 180 nM), and block TRPM8 cation channels (IC_{50} = 210 nM) (De Petrocellis et al. 2011; Yamaori et al. 2010; see also Table 6.4). At higher concentrations, CBN can target additional CYP enzymes and TRP cation channels, as well as other receptors or enzymes, and transmitter uptake processes (Table 6.4).

6.6 Δ^9-tetrahydrocannabivarin

6.6.1 Δ^9-tetrahydrocannabivarin is a CB_2 receptor partial agonist

Δ^9-THCV (Fig. 6.1) can fully displace [^{3}H]CP55940 from specific binding sites in CB_2 receptors located in membranes obtained from hCB_2-transfected CHO cells with a potency similar to that of Δ^9-THC (Table 6.1). There is also evidence that Δ^9-THCV shares the ability of Δ^9-THC both to inhibit forskolin-induced stimulation of cyclic AMP production by hCB_2-transfected CHO cells and to stimulate [^{35}S]GTPγS binding to membranes obtained from these cells (Bolognini et al. 2010). The mean E_{max} value of Δ^9-THCV was significantly less than that of CP55940 in both these assays, evidence that it activates CB_2 receptors with less efficacy than CP55940 and is, therefore, a CB_2 receptor partial agonist. Neither compound inhibited forskolin-induced stimulation of cyclic AMP production in CHO cells that had not been transfected with CB_2 receptors.

As is to be expected for a partial agonist, the ability of Δ^9-THCV to activate CB_2 receptors seems to be influenced by the expression level of these receptors. Thus, it produced a significant stimulation of [^{35}S]GTPγS binding to hCB_2 CHO cell membranes in which CB_2 receptors were expressed at a level of 215 pmol mg^{-1}, but no detectable stimulation of such binding to cell membranes in which these receptors were expressed at the lower level of 72.57 pmol mg^{-1} (Bolognini et al. 2010). Indeed, Δ^9-THCV antagonized CP55940-induced stimulation of [^{35}S]GTPγS binding to these lower CB_2-expressing membranes (Thomas et al. 2005), an indication that Δ^9-THCV possesses the typical mixed agonist-antagonist properties of a partial agonist, inducing signs of agonism when its receptors are highly expressed, but signs of antagonism when they are less highly expressed.

It has also been found that Δ^9-THCV can stimulate [^{35}S]GTPγS binding to membranes obtained from mouse spleen, and that such stimulation is not produced by Δ^9-THCV in membranes obtained from mice from which the CB_2 receptor has been genetically deleted (Bolognini et al. 2010). Hence Δ^9-THCV can activate CB_2 receptors not only in cells that have been transfected with CB_2 receptors but also in a tissue that expresses these receptors naturally. Additionally, it has been found: (1) that like the CB_2-selective agonist, JWH-015, Δ^9-THCV can stimulate fibroblastic colony formation by bone marrow cells, and (2) that this stimulation by Δ^9-THCV is reduced by the CB_2-selective antagonist, AM630 (Scutt and Williamson 2007).

There is evidence as well that CB_2 receptors can be activated by Δ^9-THCV in vivo. This has come from experiments with mice showing: (1) that this compound resembles established CB_2 receptor agonists by displaying an ability to decrease both carrageenan-induced paw edema and signs of inflammatory pain exhibited in the formalin paw test, and (2) that both these effects of Δ^9-THCV can be attenuated by the CB_2-selective antagonist SR144528 (Bolognini et al. 2010). However, the effect of Δ^9-THCV in the second of these bioassays was opposed by the CB_1 selective antagonist, SR141716A too, and although Δ^9-THCV also suppressed carrageenan-induced hind paw hyperalgesia, this effect was attenuated by neither SR144528 nor SR141716A. In addition, Δ^9-THCV attenuated the first and second phases of formalin-induced pain behavior at a dose of 5 mg kg^{-1} i.p., but only the second of these phases at the lower dose of 1 mg kg^{-1} i.p. (Bolognini et al. 2010). This is of interest since several established CB_2-selective agonists have been found to suppress only phase 2 of the formalin test (Guindon and Hohmann 2008).

Further evidence that Δ^9-THCV can activate CB_2 receptors in vivo comes from the finding that in mice that had received intrastriatal injections of lipopolysaccharide (LPS), it can produce signs of neuroprotection similar to those produced by the CB_2-selective agonist, HU-308 (García et al. 2011). It has been found too that signs of hepatic ischemia/reperfusion injury in mice can be attenuated by Δ^8-THCV in a manner that can be opposed by the CB_2-selective antagonist, SR144528 (Bátkai et al. 2012). This investigation also showed that Δ^8-THCV and 11-hydroxy-Δ^8-THCV display similar potency to Δ^9-THCV in vitro, both as CB_2 agonists in cyclic AMP assays performed with hCB_2-transfected CHO cells, and as displacers of [^{3}H]CP55940 from specific binding sites in membranes obtained from these cells.

6.6.2 Δ^9-tetrahydrocannabivarin also targets CB_1 receptors

Δ^9-THCV can induce a complete displacement of [^{3}H]CP55940 as potently from CB_1 receptors as from CB_2 receptors (Table 6.1), and has also been found to displace [^{3}H]*R*-(+)-WIN55212 and [^{3}H]SR141716A from specific binding sites on mouse brain membranes with about the same potency as that with which it displaces [^{3}H]CP55940 from these sites (Thomas et al. 2005). Importantly, however, evidence has also emerged from both in vitro and in vivo experiments that, at doses at which it activates CB_2 receptors, Δ^9-THCV behaves as a CB_1 receptor antagonist.

Turning first to the in vitro evidence, Δ^9-THCV has been found:

- to produce significant parallel dextral shifts in the log concentration–response curves of CP55940 and *R*-(+)-WIN55212 for their stimulation of [^{35}S]GTPγS binding to mouse whole brain membranes at 1 μM (Pertwee et al. 2007; Thomas et al. 2005)
- to produce such antagonism of *R*-(+)-WIN55212 at concentrations of 100 nM to 5 μM, when this assay is performed with membranes obtained from mouse cerebellum or piriform cortex (Dennis et al. 2008)
- to share the ability of one established CB_1-selective antagonist, SR141716A, to oppose inhibition of electrically evoked contractions of mouse isolated vasa deferentia induced by cannabinoid receptor agonists such as CP55940, *R*-(+)-WIN55212, and Δ^9-THC (Pertwee et al. 1995, 2007; Thomas et al. 2005)
- to share the ability of another such antagonist, AM251, to reverse *R*-(+)-WIN55212-induced decreases of miniature inhibitory postsynaptic current frequency at mouse cerebellar interneuron–Purkinje cell synapses (Ma et al. 2008).

Interestingly, Δ^9-THCV appeared to act solely as a competitive CB_1 antagonist in the first of these investigations, but not in the second one, a difference that merits further investigation. So, too, does the finding that although the potency that Δ^9-THCV displayed as an antagonist of Δ^9-THC in mouse isolated vasa deferentia was very similar to the potency it displayed as an antagonist of *R*-(+)-WIN55212 or CP55940 in [^{35}S]GTPγS binding assays performed with mouse whole brain membranes, the potency with which it antagonized *R*-(+)-WIN55212 or CP55940 was significantly higher in vasa deferentia than in brain membranes.

When administered by itself, at concentrations of up to 10 μM, Δ^9-THCV has been found neither to stimulate nor to inhibit [^{35}S]GTPγS binding to mouse whole brain membranes (Pertwee et al. 2007). Similar results have been obtained in experiments with membranes obtained from mouse cerebellum or piriform cortex or from rat cerebral cortex (Dennis et al. 2008; Hill et al. 2010), although in those investigations Δ^9-THCV was found to exert an inhibitory effect on [^{35}S]GTPγS binding at concentrations above 10 μM. However, in contrast to these findings, Δ^9-THCV has been found to induce signs of CB_1 receptor inverse agonism at 10, 100, and 1000 nM in experiments with human CB_1 (hCB_1) CHO cells, as indicated by its ability to enhance forskolin-induced stimulation of cyclic AMP production (Bolognini et al. 2010). This effect was most likely CB_1 receptor-mediated since it was not observed in cells that had been pre-incubated with pertussis toxin, a pretreatment expected to abolish $G_{i/o}$ protein-linked receptor signaling.

Turning now to evidence that Δ^9-THCV can also block CB_1 receptors in vivo, this came initially from experiments with mice showing that, at intravenous (i.v.) doses of 0.3 and/or 3 mg kg^{-1}, both Δ^9-THCV and Δ^8-THCV opposed the ability of Δ^9-THC to induce antinociception in a mouse model of acute pain (tail-flick test), and hypothermia (Pertwee et al. 2007). When injected at a dose of 2 mg kg^{-1} i.p., Δ^9-THCV has also been found to display significant potency as an antagonist both of CP55940-induced antinociception in a rat model of acute pain (hot-plate test), and of CP55940-induced inhibition of rat locomotor activity (García et al. 2011). In addition, Δ^8-THCV, but not Δ^9-THCV, has been reported to antagonize (1) Δ^9-THC-induced immobility in the mouse ring test at 0.3 and 3 mg kg^{-1} i.v. (Pertwee et al. 2007) and (2) Δ^9-THC induced antinociception in a mouse model of visceral pain at a subcutaneously administered dose of 50 mg kg^{-1} (Booker et al. 2009). It is also noteworthy that when injected intraperitoneally at doses of 2, 3, 10, or 30 mg kg^{-1}, Δ^9-THCV shares the ability both of AM251 to suppress food consumption and body weight in nonfasted mice (Riedel et al. 2009), and of SR141716A to reduce signs of motor inhibition displayed by 6-hydroxydopamine-lesioned "parkinsonian" rats (García et al. 2011).

Although there is no doubt that Δ^9-THCV can block CB_1 receptors, there is also evidence that its in vivo administration at high doses can lead to an activation of these receptors. Thus, Gill et al. (1970) discovered that Δ^9-THCV could induce catalepsy in the mouse ring test with an intraperitoneal potency 4.8 times less than that of Δ^9-THC, and it was also found in more recent experiments that when administered to mice intravenously at doses of 3, 10, 30, and/or 56 mg kg^{-1}: (1) Δ^8-THCV could produce both antinociception in the tail-flick test and hypothermia, (2) Δ^9-THCV could produce the first but not the second of these effects, and (3) Δ^8- and Δ^9-THCV could both produce immobility in the ring test (Pertwee et al. 2007). SR141716A was found to block Δ^8- and Δ^9-THCV-induced antinociception in the tail-flick test, although not Δ^8- or Δ^9-THCV-induced immobility in the ring test or Δ^8-THCV-induced hypothermia, findings that require further investigation (Pertwee et al. 2007). Further research is also still needed to investigate why Δ^8- and Δ^9-THCV block CB_1 receptors at low doses both in vivo and in vitro, but can produce signs of CB_1 receptor activation at high doses, in vivo but not in vitro.

6.6.3 CB_1 and CB_2 receptor-independent actions of Δ^9-tetrahydrocannabivarin

At concentrations above those at which it interacts with CB_1 and CB_2 receptors as an agonist or antagonist, Δ^9-THCV has been reported to activate or block certain TRP cation channels that are also targeted by Δ^9-THC (Table 6.4). There is also evidence that Δ^9-THCV can activate GPR55 with similar potency to but greater efficacy than Δ^9-THC. This has come from experiments with human GPR55-expressing HEK293 cells in which both these phytocannabinoids were found to stimulate ERK1/2 phosphorylation at concentrations above 1 μM (Anavi-Goffer et al. 2012). It was also found in the same investigation that when administered at a concentration of 1 μM, Δ^9-THCV produced a downward shift in the log concentration–response curve of L-α-lysophosphatidylinositol for its apparent activation of GPR55 that was greater in magnitude than the downward shift produced by 1 μM Δ^9-THC.

The extent to which Δ^9-THCV interacts with other pharmacological targets remains to be established. Further research is also needed to identify the mechanisms by which this phytocannabinoid inhibits firstly, electrically-evoked contractions of the mouse isolated vas deferens, at concentrations of 10 μM or more, in an apparent CB_1 receptor-independent manner (Thomas et al. 2005), and secondly, [^{35}S]GTPγS binding to the membranes of CHO cells expressing dopamine D_2, but most probably not cannabinoid CB_1 or CB_2 receptors (Dennis et al. 2008).

6.7 Caryophyllene activates CB_2 receptors

Convincing evidence has been obtained that there is at least one non-phytocannabinoid constituent of cannabis that can activate cannabinoid receptors. This is the sesquiterpene, (*E*)-BCP (Fig. 6.1), which appears to have the ability to activate CB_2 receptors. Thus, Gertsch et al. (2008) have found that this compound can:

- displace [^{3}H]CP55940 from specific binding sites on membranes obtained from hCB_2 receptor-expressing HEK293 cells with significant potency (Table 6.1)
- inhibit forskolin-induced stimulation of cyclic AMP production by hCB_2-transfected CHO cells (EC_{50} = 1.9 μM)
- stimulate calcium release within CB_2-expressing human promyelocytic leukemia (HL60) cells (EC_{50} = 11.5 μM), but not within HL60 cells devoid of CB_2 receptor surface expression, in a manner that could be blocked by 1 μM SR144528

- induce a rapid phosphorylation of ERK1/2 in both human monocytes and CB_2-expressing HL60 cells, at a concentration of 1 μM, and in a manner that could be blocked by 1 μM SR144528
- inhibit LPS-induced-stimulation of expression of the cytokines, TNF-α and IL-1β, in human whole blood at 500 nM in a manner that could be opposed by the CB_2 receptor antagonist, AM630, at 5 μM.

At this 5 μM concentration, AM630 has also been found to oppose the ability of 10 μM (*E*)-BCP to decrease LPS-induced proinflammatory cytokine expression in a rat intestinal epithelium-derived cell line (Bento et al. 2011).

As to in vivo evidence that (*E*)-BCP can activate CB_2 receptors, this has come from experiments showing that:

- oral administration of (*E*)-BCP at doses of 5 and 10 mg kg^{-1} could induce an apparent CB_2 receptor-mediated anti-inflammatory effect in mice, as indicated by its ability to attenuate intraplantar carrageenan-induced paw edema (Gertsch et al. 2008)
- an intraperitoneal (*E*)-BCP dose of 10 mg kg^{-1} could lessen the dysfunction and ameliorate the histological injury caused by cisplatin in mouse kidneys (Horváth et al. 2012)
- an orally administered (*E*)-BCP dose of 50 mg kg^{-1} could reduce signs of colitis induced in mice by dextran sulfate sodium (Bento et al. 2011).

These in vivo effects all appear to have been CB_2 receptor-mediated since the first two of them could be detected in wild-type mice, but not in mice from which the CB_2 receptor had been genetically deleted, and since the third effect was no longer produced by (*E*)-BCP if it was coadministered with a dose of AM630, 10 mg kg^{-1} i.p. or orally, that by itself did not affect dextran sulfate sodium-induced signs of colitis. It is noteworthy, however, that the PPARγ antagonist, GW9662, was also found to oppose the ability of (*E*)-BCP to inhibit these signs of colitis (Bento et al. 2011), suggesting that activation of CB_2 receptors may trigger PPARγ activation, or even that these nuclear receptors can be directly targeted by (*E*)-BCP. There is now a need for further research aimed at characterizing the pharmacology of (*E*)-BCP more fully, especially since there is already evidence that this compound is not only anti-inflammatory, but also possesses anticarcinogenic, antibiotic, antioxidant, and local anesthetic activity, as well as an ability to increase membrane permeability (Ghelardini et al. 2001; Legault and Pichette 2007).

Although (*E*)-BCP displays significant potency at displacing [^{3}H]CP554940 from specific binding sites on hCB_2 receptors, it has been found to induce only a slight displacement of this tritiated ligand from hCB_1 receptors in HEK293 cell membranes, even at the rather high concentration of 10 μM (Gertsch et al. 2008). It differs, therefore, from all other constituents of cannabis that are currently known to activate CB_2 receptors (Δ^9-THC, Δ^8-THC, CBN and Δ^9-THCV), since they all possess significant affinity for CB_1 receptors as well (Table 6.1).

6.8 Conclusions and future directions

Constituents of cannabis that have so far been found to activate cannabinoid receptors fall essentially into three pharmacological categories. These are first, the phytocannabinoids, Δ^8-THC, Δ^9-THC, and CBN, which activate both CB_1 and CB_2 receptors, second, the phytocannabinoid, Δ^9-THCV, which behaves as a CB_1 receptor antagonist at doses at which it activates CB_2 receptors, and third, the sesquiterpene, (*E*)-BCP, which can activate CB_2 receptors but lacks significant potency as a CB_1 agonist or antagonist. Further research is now required to establish whether any of the many other constituents of cannabis can activate CB_1 and/or CB_2 receptors with significant potency.

It is important to note that Δ^9-THC and Δ^9-THCV are both cannabinoid receptor *partial* agonists, since this is most probably the reason why they activate CB_2 receptors in some bioassays but block these receptors in other bioassays, and indeed, why Δ^9-THC can also behave as both an agonist and antagonist at the CB_1 receptor. Still to be investigated, however, is first, whether Δ^8-THC or CBN, which are also partial cannabinoid receptor agonists, share the ability of Δ^9-THC to both activate and block cannabinoid receptors, and second, both why Δ^9-THCV appears to activate the CB_1 receptor at doses above those at which it blocks this receptor, and why such activation is detectable in vivo but not in vitro.

At doses at or above those at which it activates CB_1 and CB_2 receptors, Δ^9-THC also interacts with a number of other pharmacological targets (Table 6.2 and 6.3). Further research is now required to identify any additional actions of Δ^9-THC, and to investigate the impact of the many cannabinoid receptor-independent actions of this phytocannabinoid on its in vivo pharmacology, for example, by seeking out any toxic or potentially beneficial effects that these other actions cause. It will also be important to characterize the non-CB_1, non-CB_2 receptor pharmacology of Δ^8-THC, CBN, Δ^9-THCV and (*E*)-BCP more fully, and to establish the extent to which the complex pharmacological "fingerprint" of Δ^9-THC overlaps with the pharmacological fingerprints of these other constituents of cannabis and indeed, of synthetic and endogenous compounds that are known to activate CB_1 or CB_2 receptors.

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Chapter 7

Known Pharmacological Actions of Nine Nonpsychotropic Phytocannabinoids

Maria Grazia Cascio and Roger G. Pertwee

7.1 Introduction

The plant *Cannabis sativa* contains more than 100 chemical compounds, known collectively as phytocannabinoids. Four of these compounds, Δ^9- and Δ^8-tetrahydrocannabinol (Δ^9- and Δ^8-THC), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), and cannabinol (CBN), can activate cannabinoid receptor type 1 (CB_1) and/or type 2 (CB_2) receptors, both in vitro at submicromolar concentrations and in vivo, and we have recently presented current information about their pharmacological actions elsewhere (Pertwee and Cascio, Chapter 6, this volume). No other phytocannabinoid investigated to date has been reported to activate CB_1 or CB_2 receptors with significant potency. These other phytocannabinoids are cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerovarin (CBGV), cannabigerolic acid (CBGA), Δ^9-tetrahydrocannabinolic acid (THCA), and Δ^9-tetrahydrocannabivarinic acid (THCVA). In this chapter we provide an overview of what is currently known about the pharmacological actions of each of these nine phytocannabinoids.

7.2 Cannabichromene (CBC)

CBC (Fig. 7.1) is, together with THC, CBD, and CBN, one of the most abundant naturally occurring cannabinoids (Brown and Harvey 1990). Even so, relatively few studies have yet been directed at identifying the pharmacological actions of this phytocannabinoid. What has been found so far is that CBC shows significant potency at targeting certain transient receptor potential (TRP) cation channels. Thus, for example, De Petrocellis et al. (2011, 2012) have reported that at concentrations below 10 μM, CBC can activate TRP ankyrin-type 1 (TRPA1) cation channels (EC_{50} = 90 nM), desensitize these channels to activation by allyl isothiocyanate (IC_{50} = 370 nM), activate TRPV4 and TRPV3 cation channels (EC_{50} = 600 nM and 1.9 μM, respectively), and desensitize TRPV2 and TRPV4 channels to their activation by an agonist (IC_{50} = 6.5 and 9.9 μM, respectively) (Table 7.1). It was also found in one or other of these investigations (Table 7.1) that CBC can, albeit with somewhat lower potency, activate TRPV1 channels (EC_{50} = 24.2 μM), desensitize TRPV3 channels to their activation by an agonist (IC_{50} = 200.8 μM), and block the activation of TRPM8 cation channels (IC_{50} = 40.7 μM). In addition, it has been reported that CBC displays an ability to inhibit both the cellular uptake of one endocannabinoid, anandamide (IC_{50} = 12.3 μM) and the metabolism by monoacylglycerol lipase of another endocannabinoid, 2-arachidonoyl glycerol (IC_{50} = 50.1 μM) (De Petrocellis et al. 2011; Tables 7.1–7.3). CBC has also been found to: (1) induce antinociception by itself and to potentiate the antinociceptive effect of THC in the mouse tail-flick assay (Davis and Hatoum 1983), and (2) stimulate the