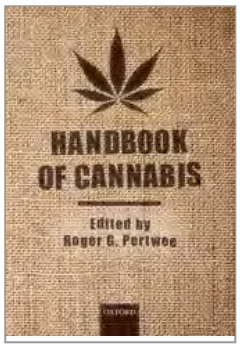

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Known Pharmacological Actions of Nine Nonpsychotropic Phytocannabinoids

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Abstract and Keywords

Cannabis is known to be the source of several pharmacologically active phytocannabinoids that do not share the ability of Δ^9-tetrahydrocannabinol, the main psychotropic constituent of cannabis, to activate cannabinoid receptors. These are cannabichromene; cannabidiol and its propyl analogue, cannabidivarin, and acid, cannabidiolic acid; cannabigerol and its propyl analogue, cannabigerovarin, and acid, cannabigerolic acid; and two other cannabinoid acids, Δ^9-tetrahydrocannabinolic acid, and its propyl analogue, Δ^9-tetrahydrocannabivarinic acid. This review describes the actions that each of these phytocannabininoids has been discovered to display to-date, in vitro, at nanomolar or micromolar concentrations, and/or in vivo. These actions include the activation, blockade or apparent allosteric modulation of certain receptors and ion channels, and the inhibition of certain enzymes and cellular uptake processes. Brief mention is also made of the ability of some of these phytocannabinoids to affect the fluidity of cell membranes, modulate cytokine release, reduce oxidative stress, and/or induce signs of neuroprotection.

Keywords: phytocannabinoids, cannabichromene, cannabidiol, cannabigerol, cannabinoid acids, propyl analogues, cannabinoid CB1 and CB2 receptors, 5-HT1A receptors, α2-adrenoceptors, GPR55, TRP cation channels

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.

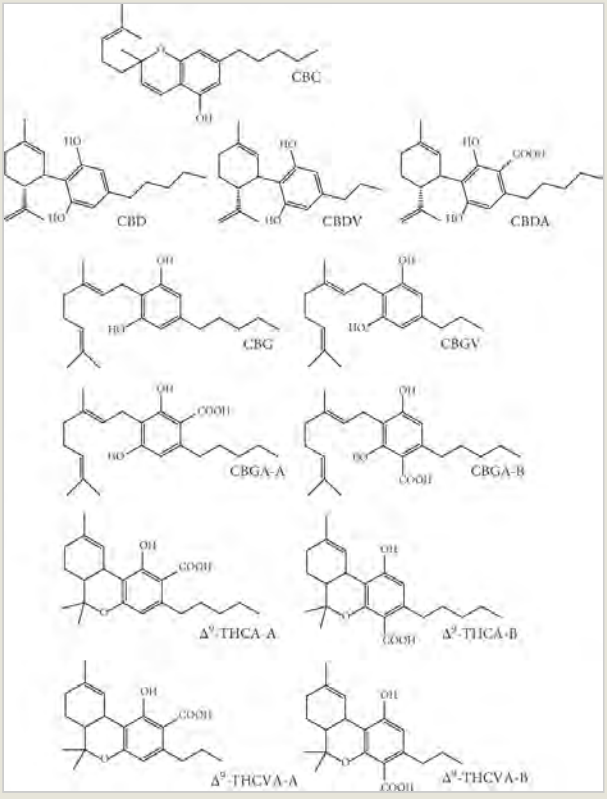

Fig. 7.1 The chemical structures of cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabigerol (CBG), cannabigerovarin (CBGV), cannabigerolic acid A (CBGA-A), cannabigerolic acid B (CBGA-B),

Δ^9-tetrahydrocannabinolic acid A (THCA-A), Δ^9-tetrahydrocannabinolic acid B (Δ^9-THCA-B), Δ^9-tetrahydrocannabivarinic acid A (Δ^9-THCVA-A), and Δ^9-tetrahydrocannabivarinic acid B (Δ^9-THCVA-B).

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 **(p.138)** descending pathway of antinociception in rat ventrolateral periaqueductal gray (Maione et al. 2011). It was also found by Maione et al. (2011) that intracerebrally injected CBC reduced tail flick-related nociception in anesthetized rats in a manner that could be blocked by intracerebral administration of the CB_1-selective antagonist, AM251, the adenosine A_1-selective antagonist, DPCPX, and the TRPA1-selective antagonist, AP18, although not by the TRPV1-selective antagonist, 5′-iodo-resiniferatoxin. The extent to which CBC induces antinociception by activating/desensitizing TRP channels, by somehow increasing the activation of adenosine A_1 receptors **(p.139)** **(p.140)** **(p.141)** **(p.142)** **(p.143)** and by activating CB_1 receptors indirectly, by elevating extracellular levels of endocannabinoids through inhibition of their cellular uptake or metabolism, remains to be established.

Table 7.1 A selection of receptors and ion channels that CBC, CBD, CBDV, or CBDA has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Receptors and channels	
CBC	< 1 µM	TRPA1 cation channel (A)	De Petrocellis et al. 2008, 2011
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
	1–10 µM	TRPV3 cation channel (A)	De Petrocellis et al. 2012
	>10 µM	TRPV1 cation channel (A)	De Petrocellis et al. 2011
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
CBD	< 1 µM	CB_1 receptor (B)	Thomas et al. 2007
		CB_2 receptor (B)	Thomas et al. 2007
		GPR55 (B)	Pertwee et al. 2010†
		$5\text{-}HT_{1A}$ receptor (P)	Rock et al. 2012
		$5\text{-}HT_{3A}$ ligand-gated ion channel (B)‡	Yang et al. 2010
		TRPM8 cation channel (B)	De Petrocellis et al. 2008, 2011
		TRPA1 cation channel (A)	De Petrocellis et al. 2008, 2011
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
	1–10 µM	CB_1 receptor (D)	Pertwee 2008†
		CB_2 receptor (D)	Pertwee et al. 2010†
		PPARγ nuclear receptor (A)	Pertwee et al. 2010†
		Ca_V3 T-type Ca^{2+} voltage gated ion channels (−)	Ross et al. 2008
		TRPV1 cation channel (A)	De Petrocellis et al. 2011

Compound and its concentration§		Pharmacological target and effect	Reference
		TRPV2 cation channel (A)	De Petrocellis et al. 2011
		TRPV3 cation channel (A)	De Petrocellis et al. 2012
		α3 glycine ligand-gated ion channel (P)	Xiong et al. 2012
	>10 μM	GPR18 (A or B)	McHugh et al. 2012
		5-HT_{1A} receptor (A)	Pertwee 2008†; Russo et al. 2005
		μ and δ opioid receptors (B)‡	Pertwee 2008†
		α1 and α1β glycine ligand-gated ion channels (P)‡	Ahrens et al. 2009
CBDV	< 1 μM	TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
	1–10 μM	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
CBDA	< 1 μM	5-HT_{1A} receptor (P)	Bolognini et al. 2013
	1–10 μM	GPR55 (B)	Anavi-Goffer et al. 2012
		TRPM8 cation channel (B)	De Petrocellis et al. 2008, 2011
		TRPA1 cation channel (A)	De Petrocellis et al. 2011

Compound and its concentration§		Pharmacological target and effect	Reference
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
	>10 µM	TRPA1 cation channel (A)	De Petrocellis et al. 2008
		TRPV1 cation channel (A)	De Petrocellis et al. 2011; Ligresti et al. 2006

Abbreviations: 5-HT, 5-hydroxytryptamine; A, activation; B, blockade; CBC, cannabichromene; CBD, cannabidiol; CBDV, cannabidivarin; CBDA, cannabidiolic acid; D, displacement of [^{3}H]CP55940 or [^{3}H]HU243 from specific binding sites; P, potentiation; PPAR, peroxisome proliferator-activated receptor; TRP, transient receptor potential; (−), inhibition or antagonism.

(†) review article;

(§) EC_{50} or IC_{50} when this has been determined;

(‡) apparent allosteric modulation.

Table 7.2 A selection of enzymes that CBC, CBD, CBDV, CBDA, CBG, CBGA, or THCA has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Enzymes	
CBC	>10 µM	Monoacylglycerol lipase (−)	De Petrocellis et al. 2011; Ligresti et al. 2006
CBD	< 1 µM	CYP1A1(−)	Yamaori et al. 2010
	1–10 µM	CYP1A2 and CYP1B1 (−)	Yamaori et al. 2010
		CYP2B6 (−)	Yamaori et al. 2011b
		CYP2C9 (−)	Yamaori et al. 2012
		CYP2D6 (−)	Yamaori et al. 2011c
		CYP3A5 (−)	Yamaori et al. 2011a

Compound and its concentration§		Pharmacological target and effect	Reference
		Mg^{2+}-ATPase (−)	Pertwee 2008†
		Arylalkylamine *N*-acetyltransferase (−)	Koch et al. 2006
		Indoleamine-2,3-dioxygenase (−)	Jenny et al. 2009
		15-lipoxygenase (−)	Takeda et al. 2009
		Phospholipase A_2 (+)	Pertwee 2008†
		Glutathione peroxidase (+)	Massi et al. 2006; Usami et al. 2008
		Glutathione reductase (+)	Massi et al. 2006; Usami et al. 2008
	>10 μM	CYP2A6 (−)	Yamaori et al. 2011b
		CYP3A4 and CYP3A7 (−)	Yamaori et al. 2011a
		Fatty acid amide hydrolase (−)	De Petrocellis et al. 2011
		Cyclooxygenase (−)	Evans 1991
		5-lipoxygenase (−)	Takeda et al. 2009
		Superoxide dismutase (−)	Usami et al. 2008
		Catalase (−)	Usami et al. 2008
		NAD(P)H-quinone reductase (−)	Usami et al. 2008
		Progesterone 17α-hydroxylase (−)	Funahashi et al. 2005; Watanabe et al. 2005
		Testosterone 6β-hydroxylase (−)	Watanabe et al. 2005
		Testosterone 16α -hydroxylase (−)	Watanabe et al. 2005
		Phosphatases (induction)	Sreevalsan et al. 2011
CBDV	>10 μM	Diacylglycerol lipase α (−)	De Petrocellis et al. 2011

Compound and its concentration§		Pharmacological target and effect	Reference
		NAAA (−)	De Petrocellis et al. 2011
CBDA	1–10 μM	Cyclooxygenase-2 (−)	Takeda et al. 2008
	>10 μM	NAAA (−)	De Petrocellis et al. 2011
		Diacylglycerol lipase α (−)	De Petrocellis et al. 2011
		Cyclooxygenase-1 (−)	Ruhaak et al. 2011; Takeda et al. 2008
CBG	1–10 μM	Lipoxygenase (−)	Evans 1991
	>10 μM	Monoacylglycerol lipase (−)	De Petrocellis et al. 2011
		Phospholipase A_2 (+)	Evans 1991
		Cyclooxygenase-2 (−)	Ruhaak et al. 2011
CBGA	>10 μM	Diacylglycerol lipase α (−)	De Petrocellis et al. 2011
		Cyclooxygenase-1 (−)	Ruhaak et al. 2011
		Cyclooxygenase-2 (−)	Ruhaak et al. 2011
THCA	>10 μM	Monoacylglycerol lipase (−)	De Petrocellis et al. 2011
		Diacylglycerol lipase α (−)	De Petrocellis et al. 2011
		Cyclooxygenase-1 (−)	Ruhaak et al. 2011
		Cyclooxygenase-2 (−)	Ruhaak et al. 2011

Abbreviations: CBC, cannabichromene; CBD, cannabidiol; CBDV, cannabidivarin;, CBDA, cannabidiolic acid; CBG, cannabigerol; CBGA cannabigerolic acid; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; THCA, Δ^9-tetrahydrocannabinolic acid. (+) activation;

(−) inhibition;

† review article.

§ EC_{50} or IC_{50} when this has been determined.

Table 7.3 A selection of cellular uptake or other processes that CBC, CBD, CBDV, CBDA, CBG, CBGA, or THCA has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Transporters and cellular uptake	
CBC	>10 μM	Cellular uptake of anandamide (−)	De Petrocellis et al. 2011; Ligresti et al. 2006
CBD	< 1 μM	Adenosine uptake by cultured microglia and macrophages (−)	Pertwee 2008†
		Synaptosomal uptake of calcium (−)	Pertwee 2008†
	1–10 μM	Synaptosomal uptake of dopamine (−)	Pertwee 2008†
		Synaptosomal uptake of norepinephrine(−)	Pertwee 2008†
		Synaptosomal uptake of 5-hydroxytryptamine (−)	Pertwee 2008†
		Synaptosomal uptake of γ-aminobutyric acid (−)	Pertwee 2008†
		Cellular uptake of anandamide (−)	De Petrocellis et al. 2011; Ligresti et al. 2006; Rakhshan et al. 2000
		P-glycoprotein (drug efflux transporter) (−)	Zhu et al. 2006
	>10 μM	Choline uptake by rat hippocampal homogenates (−)	Pertwee 2008†
CBDV	>10 μM	Cellular uptake of anandamide (−)	De Petrocellis et al. 2011
CBG	>10 μM	Cellular uptake of anandamide (−)	De Petrocellis et al. 2011; Ligresti et al. 2006
		Synaptosomal uptake of norepinephrine (−)	Banerjee et al. 1975

Compound and its concentration§		Pharmacological target and effect	Reference
		Synaptosomal uptake of 5-hydroxytryptamine (−)	Banerjee et al. 1975
		Synaptosomal uptake of γ-aminobutyric acid (−)	Banerjee et al. 1975
		Other actions or effects	
CBD	< 1 μM	Membrane fluidity (↑)	Pertwee 2004a†
	1–10 μM	Signs of neuroprotection	Fernández-Ruiz et al. 2012; Pertwee 2004a
		Oxidative stress (↓)	Pertwee 2004a†
		Release of certain cytokines (↑ or ↓)	Pertwee 2004a†
		Membrane stability (↑)	Pertwee 2004a†
	>10 μM	Release of certain cytokines (↑ or ↓)	Pertwee 2004a†

Abbreviations: CBC, cannabichromene; CBD, cannabidiol; CBDV, cannabidivarin; CBG, cannabigerol. (−), inhibition;

↑, increase;

↓, decrease;

† review article;

§ EC_{50} or IC_{50} when this has been determined.

7.3 Cannabidiol (CBD)

CBD (Fig. 7.1; Tables 7.1–7.3), was first isolated from the cannabis plant in the late 1930s and early 1940s, and its structure was elucidated in 1963 by Mechoulam and Shvo (Mechoulam and Hanus 2002). Unlike the main psychotropic component of cannabis, Δ^9-THC, CBD lacks psychotropic activity but does have therapeutic potential, both for the management of disorders such as inflammation, anxiety, emesis, and nausea, and as a neuroprotective agent and antioxidant (Pertwee 2004a, 2004b). Indeed, together with Δ^9-THC, CBD is a major constituent of Sativex®, a medicine developed by GW Pharmaceuticals that is used to ameliorate cancer pain and for the relief of neuropathic pain and spasticity due to multiple sclerosis.

7.3.1 CBD interacts with cannabinoid CB_1 and CB_2 receptors

The ability of CBD to target cannabinoid receptors has been explored in several investigations, and a brief summary of some of the assays used in that research can be found elsewhere (Pertwee and Cascio, Chapter 6, this volume). It has been found in some of these investigations that CBD displaces [^{3}H]CP55940 from cannabinoid CB_1 and CB_2 receptors at concentrations in the micromolar range (Table 7.1). In addition, in some functional in vitro assays, CBD has been found to behave as a low-potency CB_1 receptor inverse agonist as indicated by its ability at 10 μM to inhibit [^{35}S]GTPγS binding to membranes obtained either from C57BL/6 mouse brains or human CB_1-Chinese hamster ovary (hCB_1-CHO) cells (Thomas et al. 2007), or from rat cerebellum (Petitet et al. 1998). This inverse effect may or may not have been CB_1 receptor-mediated since, although CBD was found to inhibit [^{35}S]GTPγS binding to brain membranes obtained from mice from which the CB_1 receptor had been genetically deleted ($CB_1^{-/-}$ mice), it did not inhibit such binding to membranes obtained from untransfected CHO cells (Thomas et al. 2007).

Interestingly, CBD displays significant potency as an antagonist of cannabinoid receptor agonists such as CP55940 and *R*-(+)-WIN55212. Thus, there have been reports that CBD antagonizes:

- ◆ CP55940-induced stimulation of [^{35}S]GTPγS binding to rat cerebellar membranes at 10 μM (Petitet et al. 1998)
- ◆ CP55940 and *R*-(+)-WIN55212 in the mouse isolated vas deferens with apparent K_B values in the low nanomolar range (Pertwee et al. 2002)
- ◆ CP55940- and *R*-(+)-WIN55212-induced stimulation of [^{35}S]GTPγS binding to mouse brain membranes with apparent K_B values (79 and 138 nM, respectively) well below the K_i value of CBD (4.9 μM) for its displacement of [^{3}H]CP55940 from specific binding sites on these membranes (Thomas et al. 2007).

These in vitro findings are consistent with previous reports that CBD can block various in vivo responses to Δ^9-THC in rabbits, rats, mice, and human subjects (Pertwee 2004a, 2004b).

It has also been found that CBD can oppose CP55940-induced stimulation of [^{35}S]GTPγS binding to hCB_2-Chinese hamster ovary (hCB_2-CHO) cell membranes (Thomas et al. 2007). Its apparent K_B value for this antagonism was 65 nM, which is far less than its K_i value for the displacement of [^{3}H]CP55940 from such membranes (4.2 μM). CBD was also found in this investigation to inhibit [^{35}S]GTPγS binding to hCB_2-CHO cell membranes, an indication that it is a CB_2 receptor inverse agonist. Since there is convincing evidence that CB_2 receptor inverse agonists reduce **(p.144)** immune cell migration and have anti-inflammatory effects (Lunn et al. 2006), the ability of CBD to behave as a CB_2

receptor inverse agonist could account, at least in part, for its well-documented anti-inflammatory properties (Izzo et al. 2009; Pertwee 2004a, 2004b), and for its capacity to inhibit immune cell migration as demonstrated, for example, in Boyden chamber experiments performed with murine microglial cells or macrophages (Sacerdote et al. 2005; Walter et al. 2003), or with human neutrophils (McHugh and Ross 2005).

CBD is not generally regarded as being a cannabinoid receptor agonist. It is noteworthy, therefore, that there has been one report that submicromolar concentrations of this phytocannabinoid can produce a small but significant stimulation of [^{35}S]GTPγS binding to membranes obtained from CHO cells in which the hCB$_1$ receptor is highly expressed, but not to membranes obtained from CHO cells that do not express this receptor (Thomas et al. 2007). It may be, therefore, that CBD is a very low-efficacy CB$_1$ receptor partial agonist that can induce signs of CB$_1$ receptor agonism in tissues in which these receptors are highly expressed. Whether CBD can also behave in this way in vivo remains to be established. It is noteworthy, however, that there is already evidence that microsomal enzymes catalyze the metabolism of CBD to Δ^9-THC-like compounds such as 6β-hydroxymethyl-Δ^9-THC, which may well be psychotropic since it has been found to produce catalepsy, antinociception, and hypothermia in mice, albeit with less potency than Δ^9-THC (Nagai et al. 1993; Yamamoto et al. 2003). There have also been reports first, that CBD can reduce signs of compulsive behavior in mice and tail flick-related nociception in anesthetized rats in a manner that can be antagonized by the CB$_1$-selective antagonist AM251, and second, that CBD can elevate brain levels of the endogenous CB$_1$ receptor agonist, 2-arachidonoyl glycerol, in rats (Casarotto et al. 2010; Maione et al. 2011).

7.3.2 CB$_1$ and CB$_2$ receptor-independent actions of CBD

CBD has the ability to produce a large number of cannabinoid receptor-independent effects in vitro (Tables 7.1–7.3). Among these are several that it can produce at concentrations in the submicromolar range (see Tables 7.1–7.3 for references): (1) antagonism of the G protein-coupled receptor, GPR55, and of the TRP cation channel, TRPM8 (IC_{50} = 60 or 80 nM); (2) activation of TRPA1 (EC_{50} = 96 or 110 nM) and TRPV4 cation channels (EC_{50} = 800 nM), and desensitization of the TRPA1 cation channel to activation by allyl isothiocyanate (IC_{50} = 80, 140, or 160 nM); (3) desensitization of TRPV1 and TRPV3 cation channels to activation by an agonist (IC_{50} = 600 and 900 nM, respectively); (4) potentiation of the activation of the G protein-coupled 5-HT$_{1A}$ receptor and of the ligand-gated ion channel, 5-HT$_{3A}$; (5) inhibition of the human cytochrome P450 enzyme, CYP1A1; (6) inhibition of the cellular uptake of adenosine and of the synaptosomal uptake of calcium. Importantly, as indicated in sections 7.3.2.1–7.3.2.4, there is evidence that several of the in vitro effects of CBD listed earlier or in Tables 7.1, 7.2, or 7.3 can also be produced by this phytocannabinoid in vivo. There is also evidence that when administered

repeatedly to mice or rats, CBD can induce hepatic CYP3A, CYP2B10, and CYP2C enzymes (Pertwee 2004a).

7.3.2.1 Evidence that CBD can increase 5-HT$_{1A}$ receptor activation in vitro

In vitro evidence that CBD can potentiate the activation of 5-HT$_{1A}$ receptors came from the finding that it can enhance stimulation of [^{35}S]GTPγS binding to rat brainstem membranes induced by the 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Rock et al. 2012). This enhancement was found to be produced by CBD at a concentration of 100 nM, but not at concentrations of 1, 10, 31.62, or 1000 nM, indicating its concentration–response curve to be bell-shaped. This is a noteworthy finding since the dose–response curves of CBD for its production in vivo of several effects that seem to be 5-HT$_{1A}$ receptor-mediated have been found to be biphasic **(p.145)** or bell-shaped (section 7.3.2.2). It is also noteworthy that CBD did not displace [^{3}H]8-OH-DPAT from specific binding sites on rat brainstem membranes at 100 nM, or indeed, at other concentrations between 0.1 nM and 10 μM, suggesting that it did not enhance the activation of 5-HT$_{1A}$ receptors by interacting directly with sites on these receptors that are targeted by 8-OH-DPAT. Whether CBD produces this enhancement by interacting allosterically with the 5-HT$_{1A}$ receptor or by acting on a different target which then somehow augments 5-HT- or 8-OH-DPAT-induced 5-HT$_{1A}$ receptor activation through an indirect mechanism remains to be established.

7.3.2.2 Evidence that CBD can increase 5-HT$_{1A}$ receptor activation in vivo

7.3.2.2.1 CBD induces an apparent 5-HT$_{1A}$ receptor-mediated attenuation of nausea and vomiting

CBD (5 mg kg^{-1} i.p.) has been found to attenuate cisplatin-induced (Kwiatkowska et al. 2004) and lithium chloride-induced (Parker et al. 2004) vomiting and anticipatory retching (Parker et al. 2006) in shrews (*Suncus murinus*), as well as conditioned gaping (nausea-like behavior) induced by lithium chloride in rats (Rock et al. 2012). These effects of CBD all appear to be mediated by 5-HT$_{1A}$ receptors. Thus, WAY100135, a well-established 5-HT$_{1A}$ antagonist, and/or WAY100635, a more selective 5-HT$_{1A}$ antagonist, have been found to oppose the ability of CBD to reduce nicotine, cisplatin, and lithium chloride-induced vomiting in shrews, and to interfere with the establishment of lithium chloride-induced conditioned gaping in rats (Rock et al. 2011b). Moreover, when injected directly into the rat dorsal raphe nucleus: (1) WAY100635 reversed the antinausea-like effects of systemic CBD, and (2) CBD suppressed nausea-like behavior in a manner that could be opposed by systemic WAY100635 (Rock et al. 2012). It has also been found that CBD and the 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, interact synergistically to suppress nausea-like behavior in rats (Rock et al. 2012). It is noteworthy too that CBD was found to affect toxin-induced vomiting in shrews in a biphasic manner, potentiating this vomiting at doses above those at which it had a suppressant effect (Kwiatkowska

et al. 2004; Parker et al. 2004). These in vivo findings are all in line with the in vitro evidence that CBD can potentiate the activation of 5-HT_{1A} receptors, and that its concentration–response curve for the production of this potentiation is bell-shaped (section 7.3.2.1). When considered together, these in vivo and in vitro findings strongly support the hypothesis that CBD suppresses vomiting in shrews and nausea-like behavior in rats by somehow augmenting the activation of 5-HT_{1A} receptors in the brainstem by endogenously released 5-HT (Rock et al. 2012).

7.3.2.2.2 CBD induces an apparent 5-HT_{1A} receptor-mediated attenuation of cerebral infarction

CBD has been found to produce a significant reduction in infarct volume in a mouse middle cerebral artery occlusion model of cerebral infarction with a bell-shaped dose–response curve (Mishima et al. 2005). This neuroprotective effect of CBD was opposed by WAY100135, but not by the CB_1 receptor antagonist, rimonabant, or the TRPV1 antagonist, capsazepine. CBD (3 mg kg^{-1} i.p.) also increased cerebral blood flow to the cortex, and this effect too was opposed by WAY100135. These findings suggest that these effects of CBD on cerebral blood flow and infarct volume were both 5-HT_{1A} receptor-mediated.

7.3.2.2.3 CBD induces apparent 5-HT_{1A} receptor-mediated anxiolytic effects

When injected directly into the dorsolateral periaqueductal gray of rats, CBD has been found to produce signs of anxiolysis in both the elevated plus maze and the Vogel conflict test (Campos and Guimarães 2008). These effects were opposed by WAY100635, but not by AM251, supporting the hypothesis that CBD produced these apparent anxiolytic effects by targeting 5-HT_{1A} receptors in the dorsolateral periaqueductal gray. The elevated plus maze experiments were performed with several doses of CBD and the shape of the resultant dose–response curve was bell-shaped. More recently, **(p.146)** it has also been found that when CBD is injected directly into the bed nucleus of the stria terminalis of rats, it reduces both signs of anxiety in these two bioassays, and the expression of contextual fear conditioning, in a manner that can be prevented by WAY100635 (Gomes et al. 2011, 2012). Intraperitoneally administered CBD has also been found to produce signs of anxiolysis in rats, and this effect was also blocked by WAY100635 (Resstel et al. 2009). In addition, there have been reports that CBD can block panic-like responses in rats when administered intracerebrally, that its repeated intraperitoneal administration can reduce signs of anxiety in rats in a predator exposure model of posttraumatic stress disorder, and that WAY100635 antagonizes the production by CBD of both these effects (Campos et al. 2012; de Paula Soares et al. 2010).

7.3.2.2.4 Other apparent 5-HT_{1A} receptor-mediated effects produced by CBD

In experiments performed with mice, CBD has been found to share the ability of the well-established antidepressant, imipramine, to reduce immobility time in the forced swim test, without affecting exploratory behavior in an open field arena (Zanelati et al. 2010). This effect of CBD was blocked by WAY100635, suggesting that it was 5-HT_{1A} receptor-mediated. It was produced by CBD at a dose of 30 mg kg^{-1} but not by lower or higher intraperitoneal doses of this phytocannabinoid. It has also been found that repeated administration of CBD (i.p.) can induce apparent improvements in mouse locomotion and cognition following their impairment by bile duct ligation, and that these improvements can be prevented by WAY100635 (Magen et al. 2010). Finally, WAY100635 has been reported to oppose the ability of CBD to reduce tail flick-related nociception in anesthetized rats when both these compounds were injected directly into the ventrolateral periaqueductal gray (Maione et al. 2011). The dose–response curve of CBD for its production of this antinociceptive effect was bell-shaped.

7.3.2.3 Evidence that CBD can inhibit adenosine uptake both in vitro and in vivo

Release of adenosine is evoked during cellular stress and inflammation and constitutes an endogenous mechanism of immunosuppression, an effect of adenosine that is terminated by its cellular uptake and can therefore be enhanced by inhibitors of this uptake (Carrier et al. 2006). It is noteworthy, therefore, that CBD has been found to: (1) decrease the uptake of [^{3}H]adenosine into murine microglia and RAW264.7 macrophages; (2) bind to an adenosine transporter, the equilibrative nucleoside transporter 1, at submicromolar concentrations; and (3) decrease lipopolysaccharide-induced tumor necrosis factor-α production in mice in vivo in a manner that could be prevented both by an antagonist of the adenosine A_{2A} receptor and by genetic deletion of this receptor (Carrier et al. 2006). Similarly, Liou et al. (2008) have found that CBD can inhibit adenosine uptake into rat retinal microglial cells, that it can also oppose increases in tumor necrosis factor-α production in rat retina in vivo that had been triggered by lipopolysaccharide, and that this in vivo effect of CBD could be prevented by the adenosine A_{2A} receptor antagonist, ZM241385. More recently, this antagonist was also found to oppose anti-inflammatory effects induced by CBD in vivo in a mouse model of acute lung injury (Ribeiro et al. 2012). There has been a report too that the ability of intracerebrally injected CBD to reduce tail flick-related nociception in anesthetized rats can be prevented by intracerebral administration of the selective adenosine A_1 receptor antagonist, DPCPX (Maione et al. 2011).

7.3.2.4 Other actions that CBD seems to display in vivo

There is also some evidence that CBD can interact with TRPA1 and TRPV1 cation channels, α3 glycine ligand-gated ion channels, and the peroxisome proliferator-

activated receptor-γ (PPARγ) not only in vitro (Tables 7.1 and 7.2) but also in vivo.

(p.147) 7.3.2.4.1 TRP cation channels

Long et al. (2006) have found that the ability of CBD to reverse disruption of prepulse inhibition induced by MK-801 in mice in vivo could be prevented by the TRPV1 antagonist, capsazepine. It is also possible that TRPV1 activation could be at least partly responsible for the bell shape of some dose–response curves produced by CBD in vivo (e.g., see section 7.3.2.2) (Campos et al. 2012). There has also been a report that the ability of CBD injected into the ventrolateral periaqueductal gray to reduce tail flick-related nociception in anesthetized rats could be prevented by the TRPA1-selective antagonist, AP18, and, albeit less strongly, by the TRPV1 selective antagonist, 5′-iodo-resiniferatoxin, when they were injected into this brain area (Maione et al. 2011). It is noteworthy, however, that this effect of CBD was also blocked by the CB_1-selective antagonist, AM251 (section 7.3.1), the 5-HT_{1A}-selective antagonist, WAY100635 (section 7.3.2.2), and the adenosine A_1-selective antagonist, DPCPX (section 7.3.2.3).

7.3.2.4.2 Glycine ligand-gated ion channels

It has been found by Xiong et al. (2012) that genetic deletion of the α3 glycine channel, although not of the cannabinoid CB_1 or CB_2 receptor, can abolish suppression by CBD (50 mg kg^{-1} i.p.) of signs of inflammatory pain produced in mice by injecting complete Freund’s adjuvant into a hind paw.

7.3.2.4.3 Peroxisome proliferator-activated receptor-γ (PPARγ)

Esposito et al. (2011) have found that the ability of CBD (10 mg kg^{-1} i.p.) to produce neuroprotective effects in an in vivo model of Alzheimer’s disease when it was administered repeatedly could be completely prevented by the selective PPARγ antagonist, GW9662, although not by the selective PPARα antagonist, MK886. This was a model in which neuroinflammation was induced in rats by intrahippocampal injection of fibrillar Aβ peptide.

7.4 Cannabidiolic acid (CBDA)

The pharmacology of CBDA, the natural precursor of CBD in cannabis, has as yet been little investigated. What has been found so far, from in vitro experiments, is that this phytocannabinoid can target the receptor GPR55 and the cation channels TRPA1, TRPV1, and TRPM8, albeit only at concentrations between 1 and 10 μM (Table 7.1). At even higher concentrations, CBDA has also been reported to activate the cation channel, TRPV1, and to inhibit the enzymes, *N*-acylethanolamine-hydrolyzing acid amidase (NAAH) and diacylglycerol lipase α (DAGLα) (Tables 7.1 and 7.2). Consistent with the presence of a salicylic acid moiety in its structure, CBDA has, in addition, been reported be a cyclooxygenase inhibitor (Table 7.2). Thus, Takeda et al. (2008) have found that CBDA can inhibit both cycooxygenase-1 (IC_{50} = 20 μM) and cyclooxygenase-2

(IC_{50} = 2.2 μM). In contrast, CBD, which does not have a salicyclic acid moiety in its structure, did not inhibit either of these enzymes significantly even at a concentration of 100 μM. More recently, however, Ruhaak et al. (2011) reported that CBDA inhibited cyclooxygenase-1 with much lower potency (IC_{50} = 470 μM), and cyclooxygenase-2 by less than 30%, even at a concentration of 27.8 μM. Like Takeda et al. (2008), they also found CBD, and indeed Δ^9-THC, to lack significant activity for the inhibition of either of these enzymes.

In contrast, CBDA does appear to share the ability of CBD (sections 7.3.2.1 and 7.3.2.2) to display marked potency, both in vitro and in vivo, as an enhancer of 5-HT_{1A} receptor activation (Bolognini et al. 2013). Thus, in vitro experiments have shown that, at concentrations ranging from 0.1 to 100 nM, CBDA can significantly increase the maximal stimulatory effect of 8-OH-DPAT on [^{35}S]GTPγS binding to rat brainstem membranes. The dose–response curve of CBDA for the production of this effect was bell-shaped, as no such enhancement was produced by CBDA at concentrations of either 0.01 nM or 1 μM. It is also noteworthy that CBDA produced **(p.148)** this effect over a wider concentration range and with greater potency than CBD, as indicated by data obtained with CBD in a previous investigation (section 7.3.2.1). Turning now to the in vivo experiments, these showed first, that CBDA (0.01 and 0.1 mg kg^{-1} i.p.) suppressed nausea-like behavior in rats, and second, that this effect could be blocked by the selective 5-HT_{1A} receptor antagonist, WAY100635, but not by the selective CB_1 receptor antagonist/inverse agonist, SR141716A (rimonabant). Again, the dose–response curve of CBDA was bell-shaped: no antinausea effect was produced by this phytocannabinoid at doses of 0.5 or 5 mg kg^{-1} i.p. The manner in which CBDA interacted with 5-HT_{1A} receptors in these experiments remains to be established, since (like CBD) it did not displace [^{3}H]8-OH-DPAT from specific binding sites in rat brainstem membranes at concentrations ranging from 0.1 to 1000 nM. Bolognini et al. (2013) also found that, in contrast to CBD (section 7.3.1), CBDA did not display significant activity as either an agonist or an inverse agonist at cannabinoid CB_1 receptors in mouse whole brain membranes, even at a concentration 100,000-fold higher than a concentration (0.1 nM) at which it potentiated 8-OH-DPAT in rat brainstem membranes.

7.5 Cannabigerol (CBG)

CBG (Fig 7.1, Tables 7.2–7.4), is a little-investigated phytocannabinoid that has been found not to induce Δ^9-THC-like psychotropic effects in vivo (Grunfeld and Edery 1969). The structure of CBG was first established by Gaoni and Mechoulam who also performed the first synthesis of this compound (Gaoni and Mechoulam 1971). Effects that CBG has been found to produce in vitro at concentrations in the submicromolar range (Table 7.4) include:

◆ displacement of [^{3}H]CP55940 from specific binding sites on mouse brain membranes with a K_i value of 381 nM (Cascio et al. 2010)

◆ α_2-adrenoceptor agonism in both mouse brain (EC_{50} = 0.2 nM) and mouse vas deferens (EC_{50} = 72.8 nM)

◆ antagonism of the cation channel, TRPM8 (IC_{50} = 160 nM)

◆ activation of the cation channel, TRPA1 (EC_{50} = 700 nM) (De Petrocellis et al. 2011).

Table 7.4 A selection of receptors and ion channels that CBG, CBGV, CBGA, THCA, or THCVA has been reported to target in vitro

Compound and its concentration§		Pharmacological target and effect	Reference
		Receptors and channels	
CBG	< 1 μM	CB_1 receptor (D)	Cascio et al. 2010
		TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPM8 cation channel (B)	De Petrocellis et al. 2008, 2011
		α_2-adrenoceptor (A)	Cascio et al. 2010
	1–10 μM	CB_1 receptor (B)	Cascio et al. 2010
		CB_2 receptor (D)	Cascio et al. 2010
		5-HT_{1A} receptor (B)	Cascio et al. 2010
		TRPA1 cation channel (A)	De Petrocellis et al. 2008
		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
CBGV	1–10 μM	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
		TRPM8 cation channel (B)	De Petrocellis et al. 2011

Compound and its concentration§		Pharmacological target and effect	Reference
	>10 μM	TRPV4 cation channel (A)	De Petrocellis et al. 2012
CBGA	1–10 μM	TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
	>10 μM	TRPV1 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
THCA	< 1 μM	TRPM8 cation channel (B)	De Petrocellis et al. 2008, 2011
		TRPA1 cation channel (A)	De Petrocellis et al. 2008
	1–10 μM	TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPV4 cation channel (A)	De Petrocellis et al. 2012
	>10 μM	TRPV2 cation channel (A)	De Petrocellis et al. 2011
THCVA	1–10 μM	TRPV4 cation channel (A)	De Petrocellis et al. 2012
		TRPM8 cation channel (B)	De Petrocellis et al. 2011
	>10 μM	TRPA1 cation channel (A)	De Petrocellis et al. 2011
		TRPV1 cation channel (A)	De Petrocellis et al. 2011
		TRPV3 cation channel (A)	De Petrocellis et al. 2012

Abbreviations: A, activation; B, blockade; CBG, cannabigerol; CBGV, cannabigevarin; CBGA cannabigerolic acid; D, displacement of [^{3}H]CP55940 from specific binding sites; THCA, Δ^9-tetrahydrocannabinolic acid; THCVA, Δ^9-tetrahydrocannabivarinic acid; TRP, transient receptor potential. † review article;

§ EC_{50} or IC_{50} when this has been determined.

Evidence has also been obtained from in vitro experiments that CBG can oppose the activation of both CB_1 and 5-HT_{1A} receptors with significant potency. Thus, it has been found to antagonize the stimulation of [^{35}S]GTPγS binding to mouse whole brain membranes by the 5-HT_{1A} receptor agonist, 8-OH-DPAT, at 1 μM, and by the CB_1/CB_2 receptor agonists, anandamide and CP55040, at 10 μM (Cascio et al. 2010). The apparent K_B values of CBG for this antagonism, which appeared to be competitive in nature, were in the submicromolar range: 19.6 nM, 483 nM and 936 nM, for the antagonism of 8-OH-DPAT, anandamide and CP55940, respectively. There is evidence as well that CBG can activate α_2-adrenoceptors and block 5-HT_{1A} receptors when administered in vivo.

7.5.1 Evidence that CBG can activate α_2-adrenoceptors in vivo

Evidence has recently emerged that CBG can act through α_2-adrenoceptors in mice to induce signs of antinociception. Thus, Comelli et al. (2012) have reported that CBG (10 mg kg^{-1} i.p.) shares the ability of the established α_2-adrenoceptor agonist, clonidine (0.2 mg kg^{-1} i.p.), to reduce signs of persistent inflammatory pain that were induced by injecting formalin or λ-carrageenan into the hind paws of mice. The pain behavior induced by formalin usually occurs in two phases: a short, transient early phase that is followed a few minutes later by a slightly longer late phase (Guindon and Hohmann, 2008). CBG and clonidine displayed antinociceptive activity in both these phases. Importantly, at a dose of 1 mg kg^{-1} i.p., the α_2-adrenoceptor antagonist, yohimbine, significantly attenuated antinociception induced by CBG and clonidine both in the λ-carrageenan **(p.149) (p.150)** test and in the late phase, but not the early phase, of the formalin test. That CBG is able to interact with the α_2-adrenoceptor and shares the ability of clonidine to modulate pain in animals is very interesting, since the chemical structure of CBG differs greatly from that of well-established α_2-adrenoceptors ligands. Hence, CBG may constitute a lead compound for the development of a new class of α_2-adrenoceptor agonists/analgesic drugs.

7.5.2 Evidence that CBG can block 5HT_{1A} receptors in vivo

In vivo evidence that CBG is a 5-HT_{1A} receptor antagonist has come from the finding that it can prevent the 5-HT_{1A} receptor agonist, 8-OH-DPAT, from inducing antinausea effects in rats with significant potency (5 mg kg^{-1} i.p.) (Rock et al. 2011a). Since there is evidence that CBD can induce a 5-HT_{1A} receptor-mediated attenuation of both nausea in rats and vomiting in shrews (section 7.3.2.1), it is also noteworthy that CBG (5 mg kg^{-1} i.p.) has been found to prevent CBD-induced suppression of both lithium chloride-induced nausea in rats and lithium chloride-induced vomiting in shrews (Rock et al. 2011a).

7.6 Other phytocannabinoids

7.6.1 Δ^9-tetrahydrocannabinolic acid (THCA), Δ^9-tetrahydrocannabivarinic acid (THCVA), and cannabigerolic acid (CBGA)

THCA, THCVA, and CBGA (Fig 7.1, Tables 7.1 and 7.2) are the immediate natural precursors in cannabis of THC, THCV, and CBG, respectively. As indicated in Table 7.4, in vitro experiments already performed with these compounds have provided evidence that they can block the activation of TRPM8 cation channels, activate certain other TRP cation channels, or desensitize some of these channels to activation by an agonist. More specifically, there have been reports (De Petrocellis et al. 2011, 2012) that at concentrations of 10 μM or less:

- ◆ THCA blocks TRPM8 (IC_{50} = 150 nM), activates TRPA1 and TRPV4 (EC_{50} = 2.7 and 3.4 μM, respectively), and desensitizes TRPV2 and TRPV4 cation channels (IC_{50} = 9.8 and 8.8 μM, respectively)
- ◆ THCVA blocks TRPM8 (IC_{50} = 1.33 μM) and activates TRPV4 cation channels (EC_{50} = 4.4 μM)
- ◆ CBGA blocks TRPM8 (IC_{50} = 1.31 μM), activates TRPA1 (EC_{50} = 8.4 μM), and desensitizes TRPA1, TRPV3, and TRPV4 cation channels (IC_{50} = 7.14, 7.4, and 3.6 μM, respectively).

Reported effects of higher concentrations of these three phytocannabinoids on TRP cation channels are also listed in Table 7.4. In addition, THCA and CBGA, but not THCVA, have been found to inhibit DAGLα, monoacylglycerol lipase, cyclooxygenase-1, and/or cyclooxygenase-2, again at concentrations above 10 μM (Table 7.2). Finally, as indicated in Fig 7.1, there are both A and B forms of THCA, THCVA, and CBGA, and it is not always clear whether it was the A or the B form that was used in the investigations mentioned in this section or in Tables 7.2 and 7.4.

7.6.2 Cannabidivarin (CBDV) and cannabigerovarin (CBGV)

Relatively few in vitro experiments have so far been performed with CBDV and CBGV (Fig. 7.1). These have provided evidence that both compounds can block the activation of TRPM8 cation channels, activate certain other TRP channels, and desensitize some of these channels to **(p.151)** activation by an agonist (Table 7.4). More specifically, it has been reported by De Petrocellis et al. (2011, 2012) that:

- ◆ CBDV and CBGV can block the activation of TRPM8 (IC_{50} = 0.9 and 1.71 μM, respectively)
- ◆ CBDV can both activate TRPA1, TRPV1, TRPV2, TRPV3, and TRPV4 (EC_{50} = 0.42, 3.6, 7.3, 1.7, and 0.9 μM, respectively), and desensitize these cation channels (IC_{50} = 1.29, 10.0, 31.1, 25.2, and 2.9 μM, respectively)
- ◆ CBGV can also activate all these TRPA and TRPV cation channels (EC_{50} = 1.6, 2.0, 1.41, 2.4, and 22.2 μM, respectively), and desensitize them (IC_{50} = 2.02, 2.3, 0.7, 0.8, and 1.8 μM, respectively).

It has been reported too by De Petrocellis et al. (2011) that CBDV, but not CBGV, can inhibit: (1) NAAA, which catalyzes the metabolic degradation of palmitoylethanolamide; (2) a second enzyme, DAGLα; and (3) the cellular uptake of anandamide, albeit in each case only at rather high concentrations (Tables 7.2 and 7.3).

7.7 Conclusions and future directions

In conclusion it is now generally accepted that three of the nine phytocannabinoids featured in this review each displays significant potency at producing at least one action that has been detected both in vitro and in vivo. These actions are:

- the potentiation of 5-HT_{1A} receptor activation by CBDA and CBD
- the inhibition of the cellular uptake of adenosine by CBD
- the activation of α_2-adrenoceptors by CBG
- the antagonism of 5-HT_{1A} receptors by CBG
- the targeting of certain TRP cation channels by CBD and CBG.

Further research is now needed to investigate the extent to which these actions could be exploited therapeutically. It could well be, for example, that in the clinic: (1) CBDA would induce a 5-HT_{1A}-mediated suppression of chemotherapy-induced nausea and vomiting, (2) CBG would induce α_2-adrenoceptor-mediated analgesia and perhaps also reduce negative signs of schizophrenia through the blockade of 5-HT_{1A} receptors, and (3) CBD might induce PPARγ-mediated neuroprotective effects in neurodegenerative disorders such as Alzheimer's disease. It will also be important to complete the pharmacological characterization, not only of the phytocannabinoids mentioned in this review, and of their metabolites, but also of the many other as yet uninvestigated phytocannabinoids that are known to be present in cannabis. Such research would advance our understanding not only of the therapeutic potential of individual phytocannabinoids, administered alone or together with one or more other phytocannabinoid or with nonphytocannabinoid, but also of the likely myriad of pharmacological effects produced by cannabis when it is self-administered either as a recreational drug or for self-medication.

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