Neuromolecular Mechanisms of *Cannabis* Action

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Abstract

Most of our current understanding of the neuromolecular mechanisms of *Cannabis* action focusses on two plant cannabinoids, THC and CBD. THC acts primarily through presynaptic CB cannabinoid receptors to regulate neurotransmitter release in the brain, spinal cord and peripheral nerves. CBD action, on the other hand, is probably mediated through multiple molecular targets.

Keywords

Δ^9-Tetrahydrocannabinol · cannabidiol · cannabinoid receptors

Abbreviations

2AG	2-Arachidonoylglycerol
2-AGE	2-Arachidonoylglycerol ether
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDV	Cannabidivarin
CBG	Cannabigerol
COX	Cyclooxygenase
DAGL	Diacylglycerol lipase
FAAH	Fatty acid amide hydrolase
GPCR	G protein-coupled receptors
MAGL	Monoacylglycerol lipase
PLC	Phospholipase C
PPAR	Peroxisome proliferator-activated receptor
THC	Δ^9-Tetrahydrocannabinol
THCA-A	Δ^9-Tetrahydrocannabinolic acid
THCV	Δ^9-Tetrahydrocannabivarin
TRPV	Transient receptor potential vanilloid family

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2.1 Introduction and Scope of this Chapter

Cannabis, like many natural products, is a complex and variable mix of metabolites, some of which are common across many plant species, such as terpenoids and flavonoids, and some of which appear to be unique to *Cannabis*. These phytocannabinoids constitute a group of C21 or C22 terpeno-phenolic constituents, with the principal constituents being acids, including Δ^9-tetrahydrocannabinolic acid, cannabidiolic acid, cannabinolic acid, cannabinodiolic acid, cannabigerolic acid and cannabichromenic acid, for review, see Andre et al. (2016). Intriguingly, the bioactivity of the acids has drawn little attention. By contrast, the decarboxylated products of the acids have enjoyed the vast majority of

E. Murillo-Rodriguez et al. (eds.), *Cannabinoids and Neuropsychiatric Disorders*, Advances in Experimental Medicine and Biology 1264, https://doi.org/10.1007/978-3-030-57369-0_2

attention from both scientific and non-scientific audiences. A particular focus has been on THC, Δ^9-tetrahydrocannabinol, which is the predominant psychoactive cannabinoid and the primary reason for the nonmedicinal consumption of *Cannabis*. This compound was first isolated from *Cannabis* preparations over 50 years ago (Gaoni and Mechoulam 1964). The second most investigated cannabinoid is CBD, cannabidiol, which lacks the psychoactivity of THC. Our understanding of the bioactivity of the remainder of the phytocannabinoids falls off a knowledge cliff.

Accordingly, this chapter reviews the targets and neural functions of the cannabinoids. We will describe the receptor targets of THC, which are well established. We will consider the evidence for the molecular targets of CBD, which are less well-established. For the wider family of phytocannabinoids, we will review the evidence for their molecular and cellular functions.

2.1.1 Neuromolecular Targets of THC: The Cannabinoid Receptors

In 1990, the orphan G protein-coupled receptor (GPCR) SKR6 cloned from rat brain was reported to respond with similar potency to THC and its lower abundance isomer Δ^8-THC, but not CBD or cannabinol (CBN), in recombinant expression (Matsuda et al. 1990). Shortly thereafter, the human orthologue was cloned from the brainstem and testes, and identified as a cannabinoid receptor with over 97% identity to the rat protein (Gerard et al. 1991). The following year saw the first endogenous cannabinoid being identified in extracts from pig brain; this arachidonic acid conjugate was termed anandamide by Will Devane and Raphi Mechoulam (Devane et al. 1992). Three years after the cloning of the first cannabinoid receptor, a second, quite distinct GPCR was cloned from the HL60 human promyelocytic leukaemia cell line (Munro et al. 1993). This was initially described as a peripheral receptor for cannabinoids and bound THC and CBN with similar affinities, anandamide with lower affinity and CBD with much lower affinity. In 1995, a second endocannabinoid was identified; this was also an arachidonic acid conjugate, 2-arachidonoylglycerol, 2AG (Mechoulam et al. 1995; Sugiura et al. 1995).

The Nomenclature and Standards Committee of the Union of Basic and Clinical Pharmacology (NC-IUPHAR) currently recognises just two cannabinoid receptors, termed as CB_1 and CB_2 (Howlett et al. 2002; Pertwee et al. 2010), corresponding to the 'central' and 'peripheral' receptors, respectively. The two GPCRs share 44% amino acid sequence homology, although this correspondence increases to 68% for the ligand-binding domains of the transmembrane regions. They belong to the rhodopsin or family A of GPCR, which signal through pertussis toxin-sensitive $G_{i/o}$ proteins and function by activating the mitogen-activated protein kinase (MAPK) family and inhibiting adenylyl cyclase (Howlett et al. 2002; Pertwee et al. 2010).

2.1.1.1 CB_1 Cannabinoid Receptor Characterisation: Protein, Distribution, Signalling and Pharmacology

The CB_1 receptor, coded in humans by the *CNR1* gene (Pertwee et al. 2010), is of relatively long length for the rhodopsin family, 472 amino acids, having an N-terminus over 110-amino acid-long (Gerard et al. 1991). The N-terminus contains two asparagine residues, Asn^{77} and Asn^{83}, which are putative locations for glycosylation, a feature of most, if not all, GPCR. For the rat receptor, glycosylation increases the apparent molecular size from 53 to 64 kDa (Song and Howlett 1995). A similar phenomenon has been reported for the CB_1 receptor in human brain preparations (De Jesus et al. 2006). Towards the C-terminus, Cys^{415} has been described to be palmitoylated (Oddi et al. 2012), a common but not universal post-translational modification for GPCR. Palmitoylation was reported to anchor the receptor in lipid rafts of the plasma membrane and to assist in coupling to G proteins (Oddi et al. 2012). Two N-terminal splice variants of the CB_1 cannabinoid receptor have been described that differ in length and possess different ligand-binding

properties (Ryberg et al. 2005) and are expressed at significantly lower levels in various tissues (Ryberg et al. 2005; Shire et al. 1995) when compared to the full-length receptor. The physiological and pharmacological effects of these genetic variants are yet to be fully elucidated.

The first CB_1 receptor antagonist identified was rimonabant (Rinaldi-Carmona et al. 1995), which gained approval for the treatment of metabolic disorder/obesity for a brief period in Europe (Di Marzo and Despres 2009). The antagonist structure was modified to produce AM6538, which was recently crystallised with the CB_1 receptor (Hua et al. 2016). A further structurally-unrelated antagonist, taranabant, was co-crystallised in a contemporaneous study (Shao et al. 2016). Two additional crystal structures of the CB_1 cannabinoid receptor have been reported where agonists were involved. AM841 and AM11542 are structural analogues of THC (Hua et al. 2017). In an attempt to gain further understanding of the signalling mechanisms of the CB_1 receptor, the structure of a CB_1-Gi signalling complex bound to a high affinity, high efficacy agonist, MDMB-Fubinaca (Krishna Kumar et al. 2019). This characterisation of the distinct structures of the CB_1 receptor by various classes of compounds will likely aid future drug discovery centred on the cannabinoid receptors (Krishna Kumar et al. 2019).

As identified above, the CB_1 cannabinoid receptor is $G_{i/o}$-coupled, which is associated with the inhibition of cyclic AMP production (Howlett et al. 2002). In neuronal cells, however, alternative signalling pathways are thought to predominate. Thus, the CB_1 receptor couples to the potassium channel opening leading to cellular hyperpolarisation, while inhibiting voltage-gated calcium channels (Mackie et al. 1995). In recombinant expression and in particular cells in culture, the CB_1 receptor also enhances intracellular calcium release via the G protein-dependent (apparently Gβγ subunit) stimulation of phospholipase C-β (PLC-β) leading to inositol-1,4,5-trisphosphate generation (Lauckner et al. 2005).

Investigations utilising immunocytochemistry, quantitative autoradiography, and in situ hybridisation (Howlett et al. 2002) revealed that the CB_1 receptors are expressed abundantly at the terminals of central and peripheral neurons, where they inhibit the release of multiple neurotransmitters (Pertwee et al. 2010). There is high expression specifically in the cerebellum, olfactory bulb, neocortex, basal ganglia, brain stem and hippocampus (Herkenham et al. 1991). Peripherally, the CB_1 receptor is expressed in the testes, vascular endothelium, spleen, in the enteric nervous system of the gastrointestinal tract (Izzo and Sharkey 2010), adipocytes and retina.

The physiological role of CB_1 receptors in the CNS is best understood in the Schaffer collateral commissural pathway. Modelling of the operation of endocannabinoids at a glutamatergic synapse highlighted the post-junctional location of ligand-gated ion channels and G_q-coupled GPCR activated by high-frequency stimulation-evoked release of high levels of presynaptic glutamate. These receptors evoke generation of diacylglycerol (and inositol 1,4,5-trisphosphate), which is metabolised by perisynaptic diacylglycerol lipase to produce 2AG. By as yet unidentified mechanisms, 2AG leaves the postjunctional neuron to activate presynaptic CB_1 receptors, leading to the inhibition of neuronal excitability (Zachariou et al. 2013). This adaptation of synaptic efficiency is termed short-term depression or depolarisation-evoked suppression of excitation. It is attractive to hypothesise that this phenomenon is related to the observation that *Cannabis* and THC elicit impairments of short-term memory in vivo (Melges et al. 1970).

Endocannabinoids also play a role in a related phenomenon termed as depolarisation-evoked suppression of inhibition. This phenomenon plays out in much the same way as depolarisation-evoked suppression of excitation, except that the 2AG-generated post-junctionally acts on CB_1 receptors on a GABAergic nerve terminal. This leads to a reduction of GABA release, which thereby elicits disinhibition of synaptic efficacy, and enhanced neurotransmission through the affected pathway. There appears to be a predominance of CB_1 receptors on GABAergic nerve terminals compared to glutamatergic nerve terminals (Marsicano and Lutz 1999), which makes it likely that the

administration of *Cannabis* or THC leads to changes in GABA signalling pathways.

Prolonged exposure to THC has been found to produce an array of effects in humans, including analgesia, dysphoria, dependence and tolerance (Mechoulam and Parker 2013) and the majority of these effects were prevented following pretreatment with the CB_1 selective blocker rimonabant (Kendall and Yudowski 2016). In animal models, long-term treatment with THC resulted in a region-dependent reduction in the CB_1 receptor radioligand binding (Romero et al. 1997). In the hippocampus, long-term potentiation (a corollary of learning and memory) exhibited a long-lasting inhibition with repeated THC administration, persisting for up to 14 days after treatment was halted (Hoffman et al. 2007).

2.1.1.2 CB2 Cannabinoid Receptor Characterisation: Protein, Distribution, Signalling and Pharmacology

The CB_2 receptor is coded by the *CNR2* gene (Pertwee et al. 2010) located on chromosome 1p36 and is composed of 360 amino acids in humans (Munro et al. 1993). Compared to the CB_1 cannabinoid receptor, the CB_2 receptor has a much shorter N terminus, with a base molecular size of ~42 kDa, which was increased to ~55 kDa by glycosylation (Zhang et al. 2007). Analogous to the CB_1 cannabinoid receptor, a cysteine residue is expressed in the C-terminus close to the seventh transmembrane domain, Cys^{320}. As yet, there are no published data on the possibility that Cys320 is palmitoylated. In 2009, a second splice variant of the CB_2 receptor was recognised based on a human neuroblastoma cDNA library (Liu et al. 2009). The two CB_2 receptor isoforms were found to display tissue-specific expression patterns. The previously identified CB_2 receptor isoform was primarily identified in the spleen and the immune system, while the novel isoform was recognised abundantly in the testes and brain regions of the reward system.

Very recently, a crystal structure of the CB_2 receptor was published and described (Li et al. 2019). In this report, a ligand derived from rimonabant was used, which was modified to enhance CB_2 receptor affinity, AM10257. An interesting feature of the crystal structure is that the antagonist-bound conformation of the CB_2 receptor has more similarity to the agonist-bound conformation of the CB_1 receptor than the antagonist-bound version (Li et al. 2019). It will be interesting to see the impact that this divergence in structure will have on future drug design.

Both the CB_1 and CB_2 receptors share some common pharmacology (activation by the same endocannabinoids and THC) and both couple to the same family of G proteins; however, they differ profoundly in their signalling profiles. As opposed to the CB_2 receptor, the CB_1 receptor has been reported to couple in addition to Gs and to stimulate adenylate cyclase activity (Glass and Felder 1997). In early studies, comparing the two receptors in recombinant expression in the same host cells, there was a further distinction between the two receptors. When expressed in anterior pituitary cells, both receptors coupled to the inhibition of cAMP production, while only the CB_1 receptor coupled to the inhibition of voltage-gated calcium channels and opening of potassium channels (Felder et al. 1995).

Investigations using in situ hybridisation, northern blot and receptor autoradiography (Howlett et al. 2002; Pertwee 1997) revealed that the CB_2 receptor was predominantly expressed in the macrophages, spleen (Munro et al. 1993), tonsils (Carayon et al. 1998) and immune cells. The precise immune cells that abundantly express CB_2 include monocytes, B cells, polymorphonuclear neutrophils, natural killer cells, CD4+ and CD8+ T cells and when stimulated, they regulate immune cell migration and cytokine release (Galiegue et al. 1995; Schatz et al. 1997). Using quantitative PCR, the CB_2 receptor was also detected in the monocytes and macrophages of the spleen and certain leukocyte populations, precisely the eosinophils (Galiegue et al. 1995). CB_2 receptor expression was also evaluated in other human organs and it was determined that the receptor was absent from the majority of non-immune organs with the exception of the uterus, pancreas and lungs, which exhibited low levels of mRNA (Turcotte et al.

2016). The CB_2 receptor was detected in the reproductive tissues of both sexes (Battista et al. 2012; Grimaldi et al. 2009) and was suggested to perform a function in affecting the fertility of both males and females. As mentioned above, the CB_2 receptor is often referred to as the peripheral cannabinoid receptor, given its abundant peripheral presence (Howlett et al. 2002), compared to its limited CNS expression (Gong et al. 2006). Nevertheless, recent investigations have detected the expression of the CB_2 receptor in the CNS, by the microglia (Atwood and Mackie 2010) following neuroinflammation, degeneration (Ashton and Glass 2007), in neuropathic pain (Zhang et al. 2003) in multiple sclerosis and amyotrophic lateral sclerosis (Yiangou et al. 2006). The expression level of the CB_2 receptor was variable and determined by the state of the cell; i.e. microglia do not express CB_2 in healthy human brain (Stella 2004). The degree of CB_2 receptor expression in the neurons and their physiological role remains to be fully elucidated.

Presumably because of the immune location of CB_2 receptors, there are fewer investigations of the potential for tolerance with repeated administration in humans. In preclinical models, however, CB_2 receptor-selective agonists failed to exhibit tolerance in a chronic pain model (Romero-Sandoval et al. 2008). Intriguingly, pregnancy seemed to influence CB receptor expression in B lymphocytes, such that CB_2 receptors were down-regulated, while CB_1 expression was increased (Wolfson et al. 2016).

2.1.2 Neuromolecular Targets of THC: Beyond the Cannabinoid Receptors

Other than the well-identified and investigated CB_1 and CB_2 receptors, other GPCRs, ion channel and nuclear receptors have been described to be stimulated by cannabinoid ligands (Pertwee et al. 2010). There are three GPCRs, which have been described as cannabinoid foster children, rather than orphan receptors; these are GPR18, GPR55 and GPR119 (Irving et al. 2017). Although there is little sequence homology with CB_1 or CB_2 receptors (given the low homology between the CB_1 and CB_2 receptors, this may not have a deeper implication), there is some homology between the putative endogenous ligands thought to activate them. Thus, GPR119 is activated by monounsaturated fatty acid analogues of anandamide and 2AG, N-oleoylethanolamine and 2-oleoylglycerol (Overton et al. 2006), but does not appear to respond to plant-derived or synthetic cannabinoids. GPR18 and GPR55, however, have been suggested to be targets for these agents. GPR18 is proposed to be activated endogenously by an oxidised version of anandamide, N-arachidonoylglycine (Kohno et al. 2006), while GPR55 is proposed to be activated endogenously by a conjugated version of 2AG, lysophosphatidylinositol (Oka et al. 2009). GPR18 has also been reported to be activated in vitro by THC (McHugh et al. 2012), as has GPR55 (Lauckner et al. 2008). However, whether these receptors are targets for THC in vivo has not yet been determined, and the role of these receptors in neural pathways is also unclear.

The endocannabinoid anandamide (and other endogenous analogues) has also been observed to activate the TRPV1 receptor (Alharthi et al. 2018; Zygmunt et al. 1999), which is a target for the hot component of spicy food derived from chilli peppers, capsaicin (Voets et al. 2004). The TRPV1 receptor is expressed at high levels in primary afferent neurones, where it functions as a broad integrator of nociceptive signalling and is the best investigated of a large family of cation-gating ion channels, the transient receptor potential family. THC appears not to activate the TRPV1 (De Petrocellis et al. 2011), but has been observed to activate other family members: TRPA1 (De Petrocellis et al. 2008), TRPC1 (Rao and Kaminski 2006), TRPM8 (De Petrocellis et al. 2008), TRPV2 (De Petrocellis et al. 2011), and TRPV3 (De Petrocellis et al. 2012). The contribution of these interactions to the action of THC in vivo is not clear.

Both anandamide and THC have been reported to act as positive allosteric modulators of the ligand-gated ion channel glycine receptor (Hejazi et al. 2006). There is evidence that this

mechanism can contribute to the anti-nociceptive profile of these agents in vivo (Xiong et al. 2011). THC also inhibited human recombinant 5-HT_3 receptors, another ligand-gated ion channel, in vitro with high potency and efficacy (Barann et al. 2002). In contrast, THC also inhibited a third ligand-gated ion channel, α7 nicotinic acetylcholine receptors, but with a much lower potency and efficacy (Oz et al. 2004).

In terms of voltage-gated ion channels, THC was found to inhibit a number of human recombinant subtypes of voltage-gated calcium channels in vitro (Ross et al. 2008) and to inhibit an intrinsic sodium current in mouse neuroblastoma cells (Turkanis et al. 1991). Although it is attractive to hypothesise that these effects might contribute to the analgesic effect induced by THC in vivo, there is little evidence for this.

THC could be described as having an opportunistic nature in which it has a wide interactome. It has an unusual property in common with anandamide in which it is able to activate members of three of the four receptor superfamilies. Identified above are examples of GPCR and ligand-activated ion channels, which THC activates. It has also been described to activate members of the nuclear hormone receptor superfamily, particularly members of the peroxisome proliferator-activated receptors, PPARs, for review, see (O'Sullivan 2016). THC was an agonist in a reporter gene assay of PPARγ (O'Sullivan et al. 2005), although there are contrasting reports of THC action at PPARα (Sun et al. 2007; Takeda et al. 2014). PPARβ has been less thoroughly investigated in terms of responses to cannabinoids (O'Sullivan 2016).

2.1.3 Neuromolecular Targets of CBD

In contrast to THC, our knowledge of the neuromolecular mechanisms of cannabidiol is limited, which is a frustration. On the one hand, there are a number of putative targets through which CBD has been proposed to act, but none of these have features that are totally convincing as mechanisms of the in vivo action of CBD.

CBD action at the conventional cannabinoid receptors is contradictory. The majority of studies have failed to show occupancy of CB_1 or CB_2 receptors by CBD (Matsuda et al. 1990; Munro et al. 1993). However, CBD has been suggested to be a negative allosteric modulator of the CB_1 receptor in recombinant expression (Laprairie et al. 2015). There are reports that CBD can reduce the side effects caused by the administration of THC (Mechoulam and Hanus 2002) and it is attractive to hypothesise that the negative allosteric modulation of the CB_1 receptor might be the neuromolecular mechanism for this observation. CBD seems to have an interaction with the endocannabinoid system in which acute administration increased brain levels of a variety of N-acylethanolamines, including anandamide, with little impact on 2AG levels (Leishman et al. 2018). CBD has been reported to inhibit the anandamide hydrolysis enzyme fatty acid amide hydrolase activity in vitro with a range of mostly lower potencies (Bisogno et al. 2001; De Petrocellis et al. 2011; Watanabe et al. 1996) and it would be attractive to suggest this as an in vivo mechanism of action. However, the pattern of metabolite accumulation evoked by CBD and a selective inhibitor of fatty acid amide hydrolase differ, suggesting a distinct impact (Leishman et al. 2018).

CBD has been reported to interact with serotonergic signalling through multiple routes (Ledgerwood et al. 2011). It directly activated 5-HT_{1A} receptors (Russo et al. 2005), an effect implicated in anxiolytic effects (Campos and Guimaraes 2008), neuroprotection following hypoxia/ischemia (Mishima et al. 2005; Pazos et al. 2013), inhibition of nausea and vomiting behaviours (Rock et al. 2012) and inhibition of morphine-evoked reward (Katsidoni et al. 2013) of CBD in vivo. CBD also evoked an allosteric inhibition of 5-HT_{3A} receptors (Yang et al. 2010) in vitro, which may be mediated through accelerating the rate of receptor desensitisation (Xiong et al. 2011). However, this does not seem to be a major route for CBD effects in vivo.

As with THC, CBD has been reported to enhance glycine receptor function as a positive allosteric modulator (Ahrens et al. 2009), which

appeared to be mediated through a transmembrane domain-located serine residue (Foadi et al. 2010). The analgesic effects of CBD in animal models have been suggested to be mediated through glycine receptors (Lu et al. 2018; Xiong et al. 2012).

As mentioned above for THC, CBD has a broad interactome. It also has been reported to modulate the function of the transient receptor potential family to stimulate TRPA1, inhibit TRPM8 (De Petrocellis et al. 2008), stimulate TRPV1 (Costa et al. 2004), TRPV2 (Qin et al. 2008), TRPV3 and TRPV4 (De Petrocellis et al. 2012). Other investigations reported that CBD decreases neuronal hyperactivity in epilepsy by causing activation followed by rapid desensitisation of TRPV1 and TRPV2 (Iannotti et al. 2014).

CBD also regulates calcium homeostasis in the hippocampal neurons as well as blocking the low-voltage T-type calcium channels, which are prominent modulators of neuronal excitability which specifically controlled partial or generalised seizures (Jones et al. 2010).

CBD was also reported to enhance adenosine signalling by inhibiting its uptake, which has been associated with its anti-inflammatory, neuroprotective and immunosuppressive roles (Carrier et al. 2006). Additionally, this mechanism for elevating extracellular adenosine leading to an indirect activation of adenosine receptors was implicated in CBD effects in vivo on pain modulation (Maione et al. 2011), and hypoxia/ischemia-induced brain damage (Castillo et al. 2010) and ventricular arrhythmias (Gonca and Darici 2015).

2.1.4 Neuromolecular Targets of Other Cannabinoids

A number of the other cannabinoids from the Cannabis plant have been described to have effects both in vitro and in vivo. However, there is a limited capacity to correlate the two profiles.

2.1.4.1 Δ^9-Tetrahydrocannabidivarin

Δ^9-Tetrahydrocannabidivarin (THCV) is a close structural analogue of THC, which also binds to CB receptors. Initially, THCV was suggested to act as an antagonist at both CB_1 and CB_2 receptors (Thomas et al. 2005), although later it was observed to act as a partial agonist at human recombinant CB_2 receptors (Bolognini et al. 2010). CB_2 activation appeared to translate to in vivo models of inflammation (Bolognini et al. 2010) and Parkinson's disease (Garcia et al. 2011). THCV was also described to potentiate $5\text{-}HT_{1A}$ receptor function in vitro and in an in vivo model of psychosis (Cascio et al. 2015). THCV also activated TRPA1, TRPV1, TRPV2 and blocked TRPM8 channels in recombinant expression (De Petrocellis et al. 2011). In vivo, THCV was able to reverse the effects of THC in a model of visceral pain (Booker et al. 2009).

2.1.4.2 Cannabinol

Cannabinol accumulates over time by the natural oxidation of THC. It was not thought to bind the CB_1 cannabinoid receptor (Matsuda et al. 1990), but a later report described agonist action at both CB_1 and CB_2 receptors (Rhee et al. 1997). Cannabinol was less potent than THC at the CB_1 receptor, but more potent than THC at the CB_2 receptor. In vivo, cannabinol evoked a reduction in pain behaviours in a model of visceral pain in a manner sensitive to a CB_1 receptor antagonist (Booker et al. 2009) and also evoked a CB_1 antagonist-sensitive increase in feeding behaviours (Farrimond et al. 2012).

Cannabinol activated TRPA1, inhibited TRPM8, was ineffective at TRPV1, inhibited TRPV2 (De Petrocellis et al. 2011) and showed limited agonist activity at TRPV3 and TRPV4 (De Petrocellis et al. 2012).

2.1.4.3 Δ^9-Tetrahydrocannabinolic Acid

Δ^9-Tetrahydrocannabinolic acid, THCA-A, is the naturally-occurring precursor of THC, which is abundant in the *Cannabis* plant. In binding studies, THCA-A was much weaker than THC at CB_1 or CB_2 cannabinoid receptors (McPartland et al. 2017). THCA-A was less potent than THC as an

agonist at TRPA1, TRPV2, and TRPV3, as inactive at TRPV1, more active than THC at TRPV4 and equipotent as a TRPM8 antagonist (De Petrocellis et al. 2011; De Petrocellis et al. 2012). THCA was recently described as a potent PPARγ agonist in vitro, with beneficial effects in a seizure model in vivo, which could be reversed by a PPARγ antagonist (Nadal et al. 2017).

2.1.4.4 Cannabidivarin

Cannabidivarin is a close structural analogue of CBD, which displayed low-potency binding to CB_1 receptors (Hill et al. 2013), but showed sub-micromolar affinity at human recombinant CB_2 receptors (Rosenthaler et al. 2014). CBDV was a potent agonist at TRPA1, TRPV1, TRPV2, TRPV3 and TRPV4 and a potent antagonist at TRPM8 (De Petrocellis et al. 2011; De Petrocellis et al. 2012). In vivo, CBDV showed an anticonvulsant action through an unestablished mechanism (Amada et al. 2013; Hill et al. 2012; Hill et al. 2013). In addition, CBDV delayed memory deficits in mutant mice, again through an unidentified mechanism (Zamberletti et al. 2019).

2.1.4.5 Cannabidiolic Acid

Cannabidiolic acid, CBDA, is the naturally-occurring precursor of CBD. CBDA has been suggested to inhibit COX-2 (Takeda et al. 2008). CBDA was a low-potency agonist at TRPA1, less potent at TRPV1 and TRPV4, inactive at TRPV2 and TRPV3 and a low-potency antagonist at TRPM8 (De Petrocellis et al. 2011; De Petrocellis et al. 2012). CBDA antinociceptive behavioural effects were blocked by a TRPV1 antagonist in vivo (Rock et al. 2018). CBDA evoked an inhibition of nausea and vomiting behaviours in vivo; effects of which were reduced by 5-HT_{1A} receptor blockade (Bolognini et al. 2013). In vitro, CBDA appeared to act as a positive allosteric modulator of 5-HT_{1A} receptors (Bolognini et al. 2013).

2.1.4.6 Cannabigerol

Cannabigerol, CBG, has a distinct chemical structure from the other *Cannabis*-derived metabolites, with lower affinity at CB_1 and CB_2 receptors than THC (Rosenthaler et al. 2014). In contrast, it showed much higher affinity as an agonist at α_2-adrenoceptors and antagonist at 5-HT_{1A} receptors (Cascio et al. 2010). CBG was a potent agonist at TRPA1, less potent at TRPV1, TRPV2, TRPV3 and TRPV4 and a potent antagonist at TRPM8 (De Petrocellis et al. 2011; De Petrocellis et al. 2012). CBG was also able to activate both PPARα and PPARγ in vitro (D'Aniello et al. 2019). In vivo, CBG stimulated appetite in a manner yet to be explained (Brierley et al. 2016, 2017) and blocked the anti-nausea effect of CBD (Rock et al. 2011). CBG reduced colon cancer progression in vivo in a manner consistent with TRPM8 blockade (Borrelli et al. 2014).

2.1.4.7 Cannabichromene

A further chemical class of abundant *Cannabis* metabolite is cannabichromene, CBC, which exhibits lower potency at CB_1 and CB_2 receptors than THC (Rosenthaler et al. 2014). It was a very potent TRPA1 agonist with much lower potency at TRPV1, TRPV2 and TRPM8 and intermediate TRPV3 and TRPV4 potency (De Petrocellis et al. 2011; De Petrocellis et al. 2012). CBC activation of ERK activity in adult neural stem cells could be blocked by an A_1 adenosine receptor antagonist (Shinjyo and Di Marzo 2013). In vivo, CBC appeared to have anti-inflammatory properties, which was suggested to be mediated via TRPA1 channels (Romano et al. 2013).

2.1.5 Concluding Remarks

In this chapter, we have considered the evidence for bioactivity of the major cannabinoid metabolites from the *Cannabis* plant. It is clear that, although we have been aware of the predominant molecular mechanisms of action of THC for decades, there is much less knowledge of neuromolecular mechanisms for the remainder of the cannabinoids. A further point worth making is that many of these cannabinoids appear to have contradictory effects at the molecular targets which have been identified, particularly members of the TRP receptor family. Interpreting the impact of complex mixtures of these

cannabinoids in vivo is consequently extremely difficult, complicated further by variation in pharmacokinetic profiles of these agents, an issue that has been researched only in limited detail.

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