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INVITED REVIEW

Diversity of molecular targets and signaling pathways for CBD

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Abstract

Cannabidiol (CBD) is the second most abundant component of the Cannabis plant and is known to have effects distinct from Δ^9-tetrahydrocannabinol (THC). Many studies that examined the behavioral effects of CBD concluded that it lacks the psychotomimetic effects attributed to THC. However, CBD was shown to have a broad spectrum of effects on several conditions such as anxiety, inflammation, neuropathic pain, and epilepsy. It is currently thought that CBD engages different targets and hence CBD's effects are thought to be due to multiple molecular mechanisms of action. A well-accepted set of targets include GPCRs and ion channels, with the serotonin 5-HT_{1A} receptor and the transient receptor potential cation channel TRPV1 channel being the two main targets. CBD has also been thought to target G protein-coupled receptors (GPCRs) such as cannabinoid and opioid receptors. Other studies have suggested a role for additional GPCRs and ion channels as targets of CBD. Currently, the clinical efficacy of CBD is not completely understood. Evidence derived from randomized clinical trials, in vitro and in vivo models and real-world observations support the use of CBD as a drug treatment option for anxiety, neuropathy, and many other conditions. Hence an understanding of the current status of the field as it relates to the targets for CBD is of great interest so, in this review, we include findings from recent studies that highlight these main targets.

KEYWORDS

μ-CB_1 heteromers, binding sites, cannabidiol, $G_{i/0}$ coupled receptors, ion channels

Abbreviations: 2-AG, 2 Arachidonoylglycerol; 5-HT1A, 5-hydroxytryptamine 1A receptor; [3H]8-OH-DPAT, 7-(Dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol; AEA, Anandamide; AM 251, 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide; AM 630, 1-[2-(Morpholin-4-yl)ethyl]-2-methyl-3-(4-methoxybenzoyl)-6-iodoindole; BHK, Baby hamster kidney cell line; BRET, Bioluminescence resonance energy transfer; CB_1, Cannabinoid receptor 1; CB_2, Cannabinoid receptor 2; CBD, Cannabidiol; CHO, Chinese hamster ovary cell line; CP 55940, 2-[(1R,2R,5R)-5-Hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol; D2, Dopamine receptor 2; DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin; dlPAG, dorsolateral periaqueductal gray; DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine; EEG, Electroencephalogram; EMT, Endocannabinoid membrane transporter; FAAH, Fatty acid amide hydrolase; GPCR, G-protein coupled receptor; GPR55, G-protein receptor 55; GTPγS, Guanosine triphosphate gamma S; HEK 293, human embryonic kidney 293 cell; HU 210, (6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6H,6aH,7H,10H,10aH-benzo[c]isochromen-1-ol; LPI, Lysophosphatidylinositol; M3, Muscarinic receptor 3; MAGL, Monoacyl glycerol lipase; MIA, Monoiodoacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OBX, olfactory bulbectomy mouse model of depression; PLA, Phospholipase A; PPARγ, peroxisome proliferator-activated receptor gamma; PTZ, pentylenetetrazole; rCBF, regional cerebral blood flow; RVM, rostroventral medulla; SB 366791, 4'-Chloro-3-methoxycinnamanilide; SR 141716, *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride; SR 144528, 5-(4-Chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1*H*-pyrazole-3-carboxamide; THC, Δ9-tetrahydrocanabinol; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vaniloid 1; vmPAG, ventromedial periaqueductal gray; VR1, Vanilioid receptor 1; WAY 100635, *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate; WIN 55212, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate.

1 | INTRODUCTION

The plant, *Cannabis sativa*, has been used for recreational purposes for more than 4000 years. Over 60 compounds have been identified in the plant, of which the two major pharmacologically active components are $-^9$ tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD).[1]

CBD has been shown to alter several body functions and neuronal activity. For example, CBD has been reported to improve motor activity,[2] affect depression,[3] exhibit antitumorigenic activity in vitro and in vivo,[4] anti-inflammatory effects through reduction of pro-inflammatory cytokine synthesis,[5] ameliorate lipid and glycemic parameters in Type 2 Diabetes,[6] and to reduce markers of inflammation in pancreas microcirculation in a Type 1 Diabetes mice model.[7] An interesting study in humans showed that a single high dose of CBD decreased neuronal activity in limbic and paralimbic areas of the brain leading the investigators to conclude that CBD has anxiolytic effects.[8] These results were in accordance with a study[9] reporting that a similar high dose of CBD was optimally effective in inducing anxiolytic effects in a simulated public speaking test. Finally, a number of studies have reported that CBD can reduce the anxiety and psychosis-like effects seen after THC administration, and attenuate the emotional and reward processing impairments associated with a single high dose of THC (reviewed by (Freeman et al, 2019[10]).

THC and CBD show antioxidant properties and these are thought to be due to a shared chemical structure. The hydroxyl groups and double bonds present in both molecules, contribute to increase their highest occupied molecular orbital (HOMO) value; higher HOMO values indicate a higher ability of the molecule to donate an electron, making THC and CBD powerful antioxidant molecules.[11] Moreover studies using cyclic voltammetry and a fenton reaction-based system showed that CBD could reduce hyperoxide toxicity in neurons stimulated with glutamate. The antioxidant effect of CBD, evaluated in rat cortical neuron cultures, was not affected by the presence of 500 nmol/L of the selective CB_1 cannabinoid receptor antagonist SR-141716A in an in vitro preparation of ischemic injury and was higher than the effect of other antioxidants such as α-tocopherol and ascorbate in AMPA/kainate receptor toxicity assays.[12] In agreement with these findings, Hacke et al,[13] reported that the antioxidant activity of THC and CBD in pure and mixed solutions was comparable to that of well-known antioxidants such as ascorbic acid (AA), resveratrol (Resv), and (-)-epigallocatechin-3-gallate (EGCG).

Most of the effects associated with CBD are believed to be mediated through its agonistic properties at $5\text{-}HT_{1A}$[14] and TRPV1[15] receptors (Figure 1). Furthermore, it has been argued that through different mechanisms of action, CBD can modulate neuronal activity in the dorsal periaqueductal gray, bed nucleus of the stria terminalis and medial prefrontal cortex to exert anxiolytic effects. This has been extensively reviewed by Campos et al (2012).[16] In addition to summarizing the targets for CBD described earlier, in the present review we include findings from recent studies to highlight the current status of the field.

2 | CANNABINOID SYSTEM

Early studies exploring the targets for CBD focused on the cannabinoid receptor system. This system is composed of two main receptors CB_1 and CB_2, their endogenous ligands (mainly arachidonoylethanolamide – AEA; and 2-arachidonoylglycerol - 2-AG) and the enzymes responsible for endocannabinoid synthesis, reuptake and degradation (Fatty Acid Amide Hydrolase and Mono Acyl Glycerol Lipase – FAAH, and MAGL respectively).[17] CB_1 receptors are mainly distributed in the central nervous system while the CB_2 receptors are mainly present in peripheral nerve terminals and immune cells, although evidence shows that this receptor is expressed in the brain stem (for a more detailed review, see Di Marzo et al, 2004[17]). Unlike other neurotransmitters that are synthesized and stored in vesicles, endocannabinoids are synthesized on demand, after neuronal activation, in postsynaptic terminals in a Ca^{2+}-dependent manner and activate presynaptic $G_{i/0}$ cannabinoid receptors. This molecular machinery represents a retrograde signaling mechanism model responsible for long term depression of stimulatory glutamatergic neurons, and control of short-term and long-term neuronal plasticity.[18]

Initial studies examining the molecular pharmacological properties of CBD reported that it targets the cannabinoid receptor system. CBD was found to displace binding of radiolabeled CB_1 and CB_2 cannabinoid receptor agonists ([^{3}H]CP55940 and [^{3}H] R-(+)-WIN55212, respectively) with a K_i value of 120.2 nmol/L for the CB_1 receptor and 100 nmol/L for the CB_2 receptor[19] (reviewed in Pertwee, RG; 2008[20]). Furthermore, CBD reduced the efficacy and potency of signaling by 2-AG and Δ^9-THC in cells heterologously (HEK 293A) or endogenously (STHdhQ7/Q7) expressing CB_1 receptors.[21] CBD was also found to display antagonistic activity at CB_1 and CB_2 cannabinoid receptors since it produced rightward shifts in dose response curves with CP55940- and R-(+)-WIN55212 in G protein activity assays with membranes from CHO cells expressing these receptors and from mouse brain.[22] In experiments performed with brain membranes, the mean apparent K_B values of CBD for antagonism of CP55940- and R-(þ)-WIN55212-induced stimulation of [^{35}S]GTPγS binding to these membranes are 79 and 138 nmol/L, respectively, both well below the K_i value of CBD for its displacement of [^{3}H]CP55940 from specific binding sites on these membranes.[20] Finally, Pertwee et al,[23] showed that CBD exhibited antagonistic activity at cannabinoid receptors on electrically evoked contractions of the mouse isolated vas deferens.

It is important to point out that the modulatory effects CBD exerts over the psychotomimetic actions of THC in the central nervous system[10] might come from its negative allosteric modulation of CB_1 receptors, as reported by Laprairie et al.[21] To further reinforce this molecular relation between THC and CBD, Hudson et al[24] showed that CBD reverses THC associated side-effects due to inhibition of THC-mediated ERK phosphorylation in the ventral hippocampus (vHipp) of Sprague Dawley rats, as assessed by the western blot technique. Furthermore, using the open field test, the authors observed differential effects of THC vs CBD on anxiety-like behaviours. Coadministration of THC and CBD induced a significant anxiolytic effect, with rats spending significantly greater time in the center

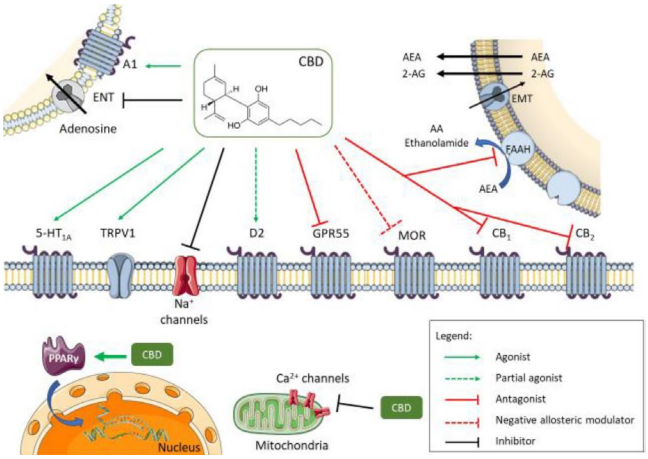

FIGURE 1 Multiple molecular targets for CBD – Cannabidiol has multiple molecular targets within the cell. It behaves as an antagonist for cannabinoid CB_1 and CB_2 receptors; however, some of the cannabinoid-mediated effects attributed to CBD may be due to its ability to inhibit endocannabinoid degradation through the FAAH enzyme. This, in turn, increases endocannabinoid levels causing receptor activation, mainly by anandamide. The full agonism at 5-HT_{1A} serotonin receptors and TRPV1 channels is responsible for the anxiolytic and analgesic effects in animals. Partial agonism at D2 dopamine receptors might account for the effects of CBD on emotional memory processing by the ventral hippocampus. Full agonism at adenosine A1 receptors might have beneficial effects on cardiac arrythmias and ischemia/reperfusion lesions in the myocardium. The negative allosteric modulation of MOR is an important CBD feature in controlling opioid drug abuse and relapse. Agonism of intracellular PPARγ receptors causes changes in gene transcription and is responsible for the positive effect of CBD on glucose and fatty acid metabolism both in animals and in humans. CBD has an overall inhibitory effect on sodium and calcium channels exerting a modulatory effect on membrane electrical potential; this would suggest CBD as a potential therapeutic for the treatment of epilepsy. CBD, cannabidiol; A1, Adenosine receptor 1; ENT, equilibrative nucleotide transporter; AEA, anandamide; 2-AG, 2-arachidonoylethanolamide; EMT, endocannabinoid membrane transporter; 5-HT_{1A}, 5-hidroxytriptamine 1A receptor; TRPV1, transient receptor potential vanilloid 1; D2, dopamine receptor 2; GPR55, G protein coupled receptor 55; MOR, μ opioid receptor; PPARγ, peroxisome proliferator-activated receptor gamma; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2

zone in relation to vehicle and THC treated groups, suggesting that intra-vHipp THC/CBD coadministration produces opposite effects on anxiety relative to THC. Moreover blockade of MEK1–2 signaling dose dependently blocks the anxiogenic effects of intra-vHipp THC, consistent with its ability to prevent intra-vHipp pERK1–2 activation.

In addition to cannabinoid receptors, CBD has also been shown to target the endocannabinoid system. CBD was found to inhibit the activity of FAAH, a major enzyme involved in anandamide hydrolysis.[15] Furthermore, the ability of CBD to inhibit AEA hydrolysis and reuptake causes an increase in the concentration of available endogenous cannabinoids to bind their respective receptors. These data are corroborated by studies by Maione et al[25] that detected increases in 2-AG in the ventromedial PAG (assessed by microdialysis) after a 3 nmol CBD microinjection. Since anandamide is the main endogenous CB_1 receptor agonist, this suggests an indirect effect of CBD on cannabinoid receptors due to increases in endogenous AEA levels. This could explain some of the cannabinoid-mediated effects attributed to CBD, even though it has been otherwise shown to be a cannabinoid receptor antagonist.[22] For example, CBD was shown to reduce inflammation in a rat model of osteoarthritis,[26] in a model of allergic contact dermatitis,[27] and in a model of experimentally inflamed explant human colonic tissue[28]; the anti-inflammatory effects could be blocked by selective CB_2 receptor antagonists. In addition, a study by Maione et al,[25] showed that CBD injected into the ventrolateral PAG induced antinociception that could be blocked by the selective CB_1 receptor antagonist, AM 251. These studies suggest agonistic activities for CBD that could be due to its ability to inhibit FAAH activity and thereby increase anandamide levels. An interesting observation was made by Massi et al,[29] who found that in vivo treatment of nude mice with CBD markedly enhanced the activity of the FAAH enzyme and reduced levels of AEA in tumor samples. These differential effects of CBD on FAAH enzyme activity could be due to differences in tissue levels of FAAH or in the different methods of assessing enzymatic activity.

A study examined the effect of CBD following direct microinjection into the ventrolateral PAG and found that this led to a reduction in the firing rate of ON and OFF cells on the rostral ventromedial medulla (RVM), and its immediate downstream neuronal circuit involved in descending pain modulation.[25] These effects were maximal with 3 nmol CBD and were antagonized by selective antagonists of cannabinoid CB_1 (AM 251), adenosine A1 (DPCPX), and TRPA1 (AP18), but not TRPV1 receptors (5′-iodo-resiniferatoxin).[25] These results support the idea that CBD functions by engaging multiple targets (Table 1).

3 | GPR55

GPR55 has been proposed to be a third cannabinoid receptor responsible for some effects attributed to cannabinoids that do not seem to be mediated through CB_1 or CB_2 receptors.[30] Ryberg et al[22] showed that cannabinoid receptor agonists such as CP55940, HU210, and Δ^9-THC can bind to and signal in heterologous cells expressing FLAG-tagged human GPR55. Like its activity at CB_1 or CB_2 receptors,[22] CBD appears to function as a GPR55 antagonist.[31] CBD decreases the potency of the agonist, CP55940, at nmol/L concentrations in a GTPγS assay with membranes from cells overexpressing GPR55.[30]

To examine the in vivo effects, a synthetic regioisomer of cannabidiol named abnormal-cannabidiol (Abn-CBD), was used; administration of Abn-CBD produced vasodilator effects, reduced blood pressure, did not have any psychotomimetic effects,[32] and showed that it could be a powerful tool to manage some of Parkinson's disease symptoms.[33] Also, Abn-CBD has an anti-cataleptic effect that is blocked by CBD confirming the agonist-antagonist activities of these two molecules at GPR55.[33]

TABLE 1 Overview of CBD molecular targets

Target	CBD Effect	Experiments/Results	References
CB_1 receptor	Antagonist	CBD decreases THC and 2-AG potencies in a GTPγS binding assay in mouse brain membranes	[22]
	Negative allosteric modulator	CBD allosterically reduces CB_1 receptor signaling in HEK 293A cells	[21]
CB_2 receptor	Antagonist	CBD decreases the potency of the receptor agonist, WIN55212, in a GTPγS assay with membranes from CHO cells overexpressing CB_2 receptors	[22]
FAAH	Inhibitor	CBD inhibits [^{14}C]-AEA hydrolysis (IC_{50} < 100 μmol/L) in N18TG2 cell membrane preparations	[15]
GPR55	Antagonist	CBD decreases the potency of the agonist, CP55940, at nmol/L concentrations in a GTPγS assay with membranes from cells overexpressing GPR55	[82]
5-HT_{1A}	Agonist	CBD displaces [^{3}H]8-OH-DPAT binding and increases G protein activity in CHO cells overexpressing the human 5-HT_{1A} receptor	[14]
	Anxiolytic-like properties	CBD increases the distance travelled in an open field test in a mouse model of depression (OBX); this is blocked by a selective 5-HT_{1A} receptor antagonist, WAY100635. CBD increases sucrose consumption in the sucrose preference test, and glutamate release as assessed by microdialysis studies	[83]
	Analgesia	Reversal of CBD-mediated analgesia by a selective 5-HT_{1A} receptor antagonist, WAY 100135, in a Von Frey filament test	[36]
Dopamine D2 receptor	Partial agonist	CBD inhibits radiolabeled domperidone binding to D2 receptors with dissociation constants of 11 nmol/L at dopamine D2High receptors and 2800 nmol/L at dopamine D2Low receptors in rat striatal membranes	[38]
Adenosine A1 receptor	Agonist	CBD induces antiarrhythmic effects against I/R-induced arrhythmias in rats; this is blocked by the adenosine A1 receptor antagonist DPCPX	[45]
Adenosine A_{2A} receptor	Agonist	Treatment with CBD (1 mg/kg) singinficantly reduces TNFα in mice challenged with LPS; this is blocked by pre-treatment with the A_{2A} adenosie receptor antagonist ZM 241385 (10 mg/kg, i.p.)	[43]
MOR and DOR	Allosteric modulator	CBD accelerates [^{3}H]DAMGO dissociation from MOR and [^{3}H]-NTI from DOR induced by 10 μmol/L naloxone or 10 μmol/L naltrindole, respectively, in cerebral cortical tissue from male Wistar rats (assessed by kinetic binding studies)	[47]
TRPV1	Agonist	CBD increases cytosolic calcium levels to the same extent as the full agonist capsaicin in HEK 293 cells overexpressing the human TRVR1 receptor.	[15]
		CBD reduces leaver pressing in a cocaine self-administration test; this is blunted by capsazepine, a TRPV1 receptor antagonist	[62]
Sodium channels	Inhibition	CBD inhibits hNav1.1-1.7 currents (IC_{50} of 1.9–3.8 μmol/L). Voltage-clamp electrophysiology in HEK-293 cells and iPSC neurons shows that CBD preferentially stabilizes inactivated Nav channel states	[63]
Calcium channels	Inhibition of L-type channels	Patch-clamp techniques show that CBD inhibits L-type Ca^{2+} channels (IC_{50} of 0.1 μmol/L) in rat myocytes.	[65]
	Bidirectional effect on Ca^{2+} levels	Mitochondrion-specific Ca^{2+} sensor, Rhod-FF, shows that CBD reduces $[Ca^{2+}]_i$ levels under high excitability conditions but causes an increase under basal conditions in hippocampal primary neuronal cultures	[66]
PPARγ receptor	Agonist	CBD induces reactive gliosis in rat primary astroglial cultures; this is significantly blunted by a selective antagonist of PPARγ receptors, GW9662	[72]
	Anti-inflammatory	CBD reduces leukocyte rolling and adhesion to the endothelium in a MIA-injected model of inflammation in rats	[26]
	Antioxidant	CBD reduces hyperoxide toxicity in neurons stimulated with glutamate (evaluated by cyclic voltammetry and a fenton reaction-based system); this is not altered by cannabinoid receptor antagonists	[12]

4 | 5-HT$_{1A}$ RECEPTORS

One of the main proposed molecular targets for CBD is the serotonin receptor 5-HT$_{1A}$. Russo et al, (2005)[14] showed that in heterologous cells expressing the 5-HT$_{1A}$ receptor, CBD produced a dose-dependent displacement of [^{3}H]8-OH-DPAT binding, a selective 5-HT$_{1A}$ agonist, and at a high dose was able to induce robust [^{35}S]GTPγS binding, supporting an agonistic activity for CBD at this receptor.[14] To further reinforce the notion that CBD is interacting with the orthosteric binding site of 5-HT$_{1A}$ receptors, the selective antagonist NAN-190 was used in the cAMP assay that assessed the percent inhibition of forskolin-stimulated cAMP levels in CHO cells. Both 5-HT and CBD reduced the percentage of forskolin-stimulated AMP in the cells, and this reduction was blocked by NAN-190. This suggests that NAN-190 is competing with 5-HT or CBD for the orthosteric binding site of the 5-HT$_{1A}$ receptor.

Behavioral studies examining the involvement of the 5-HT$_{1A}$ receptor found that CBD increased the percentage of time rats spent on the Elevated Plus Maze.[34] This response was similar to other known anxiolytic substances, such as AEA and its analogue ACEA,[35] and involved 5-HT$_{1A}$ activation in the dorso-lateral PAG as suggested by reversal of anxiolytic effects in the presence of a selective 5-HT$_{1A}$ receptor antagonist, WAY-100635.[34] Additionally, a study assessing the antidepressant effects of CBD found increased rodent vertical motor activity and that this was blunted by the 5-HT$_{1A}$ receptor antagonist, WAY 100635.[2] CBD could also potentiate the effects of 8-OH-DPAT, a selective 5-HT$_{1A}$ receptor agonist, in motor activity.[2] This supports the involvement of the 5-HT$_{1A}$ receptor in the antidepressant effects of CBD.

Studies have also examined the antiallodynic effects of CBD. A study using a rat model of neuropathic pain, streptozotocin-induced diabetes, found that CBD was able to attenuate mechanical allodynia; this was blocked by WAY 100135, a selective 5-HT$_{1A}$ receptor antagonist, but not by AM 251 or AM 630, selective CB$_1$ and CB$_2$ receptor antagonists, respectively.[36] Another study used a different model of neuropathic pain, paclitaxel-induced neuropathy, and showed that CBD could attenuate mechanical allodynia. The latter effect was blocked by the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 but not by the CB$_1$ antagonist, SR141716, or the CB$_2$ antagonist, SR144528.[37] Together these studies show that many of CBD's effects are mediated through 5-HT$_{1A}$ receptor activation in the central and peripheral nervous system, regulating neuronal excitability and neurotransmitter release.

5 | DOPAMINE RECEPTORS

CBD has been proposed as a partial agonist of D2 dopamine receptorssince it inhibits radiolabeled domperidone binding to D2 receptors with dissociation constants of 11 nmol/L for dopamine D2High receptors (dopamine D2 receptors in the high affinity state) and 2800 nmol/L for dopamine D2Low receptors (dopamine D2 receptors in the low affinity state) in rat striatal membranes.[38] Through molecular modeling (Molecular mechanics energies combined with generalized Born and surface area continuum solvation, MM-GBSA) of D2 and D3 receptors in complex with CBD and haloperidol, Stark et al,[39] showed that CBD might bind more favorably to D3 dopamine receptors compared to D2 receptors, and probably acts as a partial agonist at this receptor.

Although not acting directly at dopamine receptors, cannabinoids have been shown to alter dopamine signaling in the brain. The ventral hippocampus (VHipp) is responsible for transmitting emotionally relevant contextual information to the mesolimbic dopaminergic system thereby controlling the amount of dopamine being released at the ventro-tegmental area (VTA).[40] Systemic or intra-VHipp injection of WIN55,212–2 (CB$_1$ receptor agonist) was shown to increase VTA dopaminergic neuronal activity and bursting rates, decrease VTA non-dopaminergic neuronal activity, and elicit dopamine efflux directly into the nucleus accumbens shell. These effects were reversed by SR141716A (CB$_1$ receptor antagonist).[41] THC and CBD were shown to exert differential control over dopamine activity states and emotional memory processing because of their opposing effects on molecular signaling pathways underlying schizophrenia.[40,42]

6 | ADENOSINE RECEPTORS

CBD, alongside with THC, was shown to inhibit adenosine reuptake with an IC$_{50}$ of 124 nmol/L by acting as competitive inhibitors at the equilibrative nucleotide transporter on EOC-20 microglia cells; this increases the endogenous adenosine content available for adenosine receptor activation.[43] Furthermore, treatment with CBD (1 mg/kg) significantly reduced tumor necrosis factor (TNFα) in mice challenged with lipopolysaccharides (LPS); this was blocked by pretreatment with the selective A$_{2A}$ adenosine receptor antagonist, ZM 241385 (10 mg/Kg, i.p.).[43] The role of A$_{2A}$ adenosine receptors as CBD targets was confirmed by Ribeiro et al,[44] who found that CBD-mediated anti-inflammatory effects were reversed by the A$_{2A}$ receptor antagonist, ZM 241385, in a murine model of acute lung injury.

In a different context, CBD was shown to have antiarrhythmic effects against I/R-induced arrhythmias in rats and this was blocked by the adenosine A$_1$ receptor antagonist DPCPX,[45] indicating that CBD might activate more than one type of adenosine receptor.

7 | OPIOID RECEPTORS

The idea that cannabinoids might have modulatory effects at opioid receptors was initially postulated by Vaysse et al,[46] where they showed that Δ^9-THC decreased [3H]dihydromorphine binding to MOR due to a reduction in the number of binding sites. According to their findings, this suggests that the interaction of Δ^9-THC with opioid receptors occurs in a non-competitive manner most likely acting as a negative allosteric modulator. Investigations by Kathmann et al,[47] show that both THC and CBD at 30 μmol/L concentration behave as negative allosteric modulators of MOR and δ opioid receptors (DOR) since they accelerated the dissociation of [^{3}H]-DAMGO

(pEC_{50} = 4.67 and 4.38 for THC and CBD, respectively) and [^{3}H]-naltrindole (pEC_{50} = 5.00 and 4.10 for THC and CBD, respectively) from MOR and DOR in displacement binding assays using rat brain cortical membranes. THC increased the dissociation of [^{3}H]-DAMGO by a factor of 2; cannabidiol increased the dissociation markedly at least by a factor of 12.[47]

A study by Viudez-Martínez et al,[48] showed that administration of CBD led to a reduction in the MOR gene expression among other genes; this led the authors to speculate that CBD might be responsible for reducing the reinforcing properties, motivation and relapse for ethanol consumption in the two-bottle choice (TBC) paradigm in mice. This experimental approach is very useful for measurement of stress-induced anhedonia in mice using sucrose as a reward stimuli in one of the bottles, as opposed to water on the other.[49] It takes advantage of the fact that rodents naturally and avidly consume sweet food and selectively drink sweet drink solution when presented with a two-bottle free-choice access to both the sucrose solution and regular water. However, when exposed to stress induced models of depression, rodents failed to drink sweetened water in preference to regular water.[50–52] Thus, using this model Viudez-Martínez et al,[48] found that CBD (60 and 120 mg/kg/day, i.p.) reduced ethanol consumption and preference in the two-bottle choice in C57BL/6J mice. Moreover CBD significantly decreased ethanol intake and the number of effective responses in the oral ethanol self-administration. Parallel to that, they found that CBD significantly reduced Oprm1 gene expression, among other genes, leading the authors to conclude that CBD reduced the reinforcing properties, motivation and relapse for ethanol.

In this context, Hurd[53] shed some light on the importance of CBD as a potential tool for the treatment of Opioid Use Disorder (OUD) pointing out that CBD is not rewarding[54] and as such has limited misuse potential. Moreover CBD has remarkable positive effects on the treatment of anxiety[55] and sleep disorders,[56] major behavioral features of drug addiction, as well as a neuroprotective effect[57] making it safe to be used at high doses for the treatment of a variety of conditions.[58] With this pharmacological profile, CBD could provide a strong alternative treatment to inhibit drug-seeking behavior and curb the current opioid abuse and misuse crisis that strikes the United States and other countries.

Due to its modulatory activity over the endocannabinoid system, the close interactions between the cannabinoid and the opioid systems, its anxiolytic properties and lack of psychostimulant effects, CBD could be a powerful tool to be used in drug abuse treatments and withdrawal syndrome. For a more comprehensive review on the potential of CBD in the treatment of drug addiction, see Hurd et al (2015).[59]

8 | ION CHANNELS

A proposed molecular target for CBD is the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor (also known as VR1 receptor). A study by Bisogno et al,[15] showed that CBD can displace capsaicin from the TRPV1 receptor and increase intracellular Ca^{2+} levels to the same extent as the full agonist capsaicin in heterologous cells overexpressing TRVR1 suggesting that it functions as an agonist of this receptor.

TRP channels belonging to subfamily V type 2 (TRPV2) and subfamily A type 1 (TRPA1) have also been implicated as potential targets of CBD in modulating neuronal hyperactivity.[60] Electroencephalographic (EEG) evaluation of brain activity showed that 60 mg/kg CBD had anticonvulsant effects in a mice model of seizure induction.[61] Interestingly, CBD increased seizure latency and reduced seizure duration when injected intraperitoneally, and these effects were reversed by SB 366791, AM 251 and AM 630, selective antagonists of the TRPV1, CB_1, and CB_2 receptors, respectively.[61] This suggests an involvement of additional targets beyond the TRPV1 channel receptors, such as the endocannabinoid system, in the anticonvulsant and anti-epileptic effects of CBD.[61]

In a study using multiple models of cocaine self-administration, researchers evaluated the effects of a wide range of cocaine (0.031, 0.0625, 0.125, 0.25, 0.5, and 1 mg/kg/infusion) and CBD (3,10, 20, and 40 mg/kg, i.p.) doses on cocaine mediated reward behavior. Using different protocols of cocaine administration, such as the fixed ratio 1 (FR1 – cocaine reinforcement given after 1 attempt of self-administration) or the progressive ratio (PR – increasing response requirement for cocaine delivery over successive attempts of self-administration) schedule of reinforcement, coupled with in vivo microdialysis with high-performance liquid chromatography (HPLC) assays to evaluate brain dopamine levels, scientist showed that systemic administration of 20 mg/kg CBD dose-dependently inhibited cocaine self-administration; this was blocked by AM 630, WAY100135, and capsazepine (selective CB_2, 5-HT_{1A}, and TRPV1 receptor antagonists, respectively) demonstrating that targets beyond TRPV1 enable CBD effects.[62] Furthermore, they showed that CBD given at the dose of 20 mg/kg attenuates cocaine-induced dopamine in the nucleus accumbens, which suggests that CBD plays an important role in controlling brain response to cocaine and the consequent drug seeking behavior triggered by drug consumption.[62]

Together these studies show that CBD has modulating effects at different doses (3,10, 20, and 40 mg/kg) and routes of administration (intrapretitoneal, subcutaneous), that are mainly dependent on its agonistic activity at TRPV1 and 5-HT_{1A} receptors.[14,15] Although both receptors are responsible for several CBD-mediated actions, other targets might also be involved in distinct effects attributed to CBD and need to be further investigated.

In addition to TRPV chaanels, CBD has also been shown to engage sodium and calcium channels. CBD inhibits hNav1.1-1.7 currents, with an IC_{50} of 1.9–3.8 μmol/L in HEK-293 cells and in iPSC neurons, due to preferential stabilization of inactivated Nav channels.[63] The effects of CBD on biophysical properties such as membrane fluidity and sodium channel conductance could be responsible for its positive outcomes in the treatment of epilepsy and other hyperactivity syndromes.[64] CBD has also been shown to inhibit L-type Ca^{2+} channels with an IC_{50} of 0.1 μmol/L as detected by patch-clamp techniques in rat myocites.[65] Using mitochondrion-specific Ca^{2+} sensor (Rhod-FF,

AM), it was shown that CBD reduces $[Ca^{2+}]_i$ levels in high excitability states and increases $[Ca^{2+}]_i$ levels in control states in hippocampal primary culture cells.[66] The modulatory properties of CBD on Na^+ and Ca^{2+} channels might have a great impact on neuronal excitability. Sodium currents in peripheral neurons are mainly responsible for sensory afferent stimuli to reach the central nervous system.[67] If CBD can control part of the afferent stimuli coming from the periphery, the analgesic effects reported by Phillpot et al and Ward et al[26,68] might be due to this ability of CBD to control membrane excitability.

9 | PPARγ RECEPTORS

Peroxisome proliferator-activated receptor gamma(PPARγ) is intimately related to glucose metabolism and insulin signaling in skeletal muscle and liver.[69] Thiazolidinediones are insulin-sensitizing drugs that are greatly used to treat Type 2 diabetes to improve the metabolic profile of patients.[70] Some thiazolidinediones such as rosiglitazole and pioglitazole, have been shown to be PPARγ agonists and stimulate the transcription of insulin and fatty acid regulating genes leading to restoration of the glycemic profile in db/db mice.[71] These are obese mice due to leptin receptor knockout that have considerably higher caloric intake, hyperglycemia, dyslipidemia, and metabolic syndrome and are commonly used as models of Type 2 Diabetes and obesity.

CBD has been shown to have agonistic activities at PPARγ that might explain CBD-mediated improvements in lipid and glycemic parameters in Type 2 Diabetes.[6] Blockade of PPARγ with the selective antagonist, GW9662, significantly blunted CBD effects on reactive gliosis in rat primary astroglial cultures.[72] Moreover agonism of PPARγ by CBD might be an attractive therapeutic tool for Alzheimer's disease (AD). There is a significant body of evidence showing the efficacy of PPARγ agonists, such as pioglitazole,[73] in ameliorating disease-related pathology and improving learning and memory in animal models of AD. Recent clinical trials showed a significant improvement in memory and cognition in AD patients treated with rosiglitazone.[74] It is important to highlight that in addition to CBD, endogenous cannabinoids such as anandamide and 2-AG can also activate PPARγ and produce anti-inflammatory responses.[75]

10 | CONCLUSIONS AND PERSPECTIVES

CBD's potential as a therapeutic comes from its multiple mechanisms of action. This wide range of pharmacological activity underlies the effects of CBD on anxiety, depression, pain, memory, metabolism and more. One potential novel target is GPCR heteromers, a macromolecular complex composed of at least two functional receptor units (protomers) with biochemical properties that are demonstrably different from those of its individual components. There are three criteria for G-protein heteromers in native tissues: (a) Heteromer components should colocalize and physically interact; (b) Heteromers should exhibit properties distinct from those of the protomers; (c) Heteromer disruption should lead to a loss of heteromer-specific properties.[76] CBD has been reported to be an allosteric modulator of the DOR[47] and heteromers between the DOR and the CB_1 cannabinoid receptors have been reported.[77] Furthermore, MOR has also been shown to interact with CB_1 cannabinoid receptors.[22,47,78-81] Hence, it is possible to envision that CBD could exert some of its effects via MOR-CB_1 heteromers. By characterizing heteromer fingerprints, future studies could establish MOR-CB_1 heteromer as a target of CBD.

While it is important to recognize the beneficial effects of CBD, it is even more important to understand that it is not a miraculous drug that can be effectively used in any given pathology or condition. While the pharmacodynamic properties are being characterized, further studies need to be undertaken to better characterize the pharmacokinetic properties of CBD, the correct dosage and routes of administration for each specific condition, advantages of coadministration with other substances (especially with morphine in the context of pain management) and whether detrimental side-effects arise from chronic treatment with CBD.

11 | NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al, 2018),[82] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al, 2019).[83,84]

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DISCLOSURE

All authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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