

REVIEW

The pharmacology of cannabinoid receptors and their ligands: an overview

RG Pertwee

School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK

Mammalian tissues express at least two cannabinoid receptor types, CB₁ and CB₂, both G protein coupled. CB₁ receptors are found predominantly at nerve terminals where they mediate inhibition of transmitter release. CB₂ receptors occur mainly on immune cells, one of their roles being to modulate cytokine release. Endogenous agonists for cannabinoid receptors also exist, and are all eicosanoids. The first-discovered of these 'endocannabinoids' was arachidonylethanolamide and there is convincing evidence that this ligand and some of its metabolites can activate vanilloid VRI (TRPV1) receptors. Certain cannabinoids also appear to have TRPV1-like and/or non-CB₁, non-CB₂, non-TRPV1 targets. Several CB₁- and CB₂-selective agonists and antagonists have been developed. Antagonists include the CB₁-selective SR141716A, AM251, AM281 and LY320135, and the CB₂-selective SR144528 and AM630. These all behave as inverse agonists, one indication that CB₁ and CB₂ receptors can exist in a constitutively active state. 'Neutral' cannabinoid receptor antagonists have also been developed. CB₁ and/or CB₂ receptor activation appears to ameliorate inflammatory and neuropathic pain and certain multiple sclerosis symptoms. This might be exploited clinically by using CB₁, CB₂ or CB₁/CB₂ agonists, or inhibitors of the membrane transport or catabolism of endocannabinoids that are released in increased amounts, at least in animal models of pain and multiple sclerosis. We have recently discovered the presence of an allosteric site on the CB₁ receptor. Consequently, it may also prove possible to enhance 'autoprotective' effects of released endocannabinoids with CB₁ allosteric enhancers or, indeed, to reduce proposed 'autoimpairing' effects of released endocannabinoids such as excessive food intake with CB₁ allosteric antagonists.

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Introduction

Mammalian tissues are now known to express at least two types of cannabinoid receptor, both G-protein coupled.¹ These are CB₁ receptors, cloned in Tom Bonner's laboratory in 1990,² and CB₂ receptors, cloned by Sean Munro in 1993.³ This article provides an overview of the pharmacology of these receptors, where possible citing other review articles that provide more detailed information and list additional references.

Cannabinoid receptor signalling, distribution and functions

Both CB₁ and CB₂ receptors are coupled through G_{i/o} proteins, negatively to adenylate cyclase and positively to

mitogen-activated protein kinase. CB₁ receptors are also coupled through G_{i/o} proteins to certain ion channels, positively to inwardly rectifying and A-type outward potassium channels, and negatively to D-type outward potassium channels⁴ and to N-type and P/Q type calcium channels. CB₁ receptors can also act through G_s proteins to activate adenylate cyclase. A more detailed description of these and other signalling mechanisms that have been proposed for cannabinoid CB₁ and CB₂ receptors can be found elsewhere.¹

Although CB₁ receptors are expressed by certain non-neuronal cells and tissues, for example the pituitary gland, immune cells and reproductive tissues, they are found predominantly at central and peripheral nerve terminals where they mediate inhibition of transmitter release.¹ CB₂ receptors occur mainly on immune cells, one of their roles being to modulate cytokine release. Thus, a common role of CB₁ and CB₂ receptors appears to be the modulation of ongoing release of chemical messengers, CB₂ receptors from immune cells and CB₁ receptors mainly from neurones.¹ Interestingly, evidence is emerging that CB₁ receptors can exist as homodimers and also that they may form

Correspondence: RG Pertwee, School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK.

E-mail: rgp@abdn.ac.uk

heterodimers or oligomers with one or more other classes of coexpressed G protein-coupled receptor in a manner that may lead to cross-talk between CB₁ and non-CB₁ receptors.⁵ Indeed, it has been proposed that on-going cross-talk between presynaptic CB₁ and α_2 -adrenergic receptors may account for the ability of clonidine to potentiate inhibition of electrically-evoked contractions of the mouse isolated vas deferens by the cannabinoid receptor agonist, *R*-(+)-WIN55212.⁶

The distribution pattern of CB₁ receptors within the central nervous system accounts for several prominent effects of (-)- Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychotropic constituent of cannabis. Examples include its ability to decrease motor activity as indicated in rodents by hypokinesia and catalepsy, to induce signs of analgesia in animals and man and to stimulate food intake.^{1,7,8} Although often regarded as peripheral receptors, CB₂ receptors have been detected in the central nervous system, for example on microglial cells.^{1,5,9}

Endogenous agonists for cannabinoid receptors

The unequivocal demonstration that mammalian tissues express cannabinoid receptors was followed by the discovery that they can also produce endogenous ligands for these receptors.¹⁰ These 'endocannabinoids' are all eicosanoids, prominent examples including arachidonylethanolamide (anandamide) and 2-arachidonoyl glycerol, both of which are synthesized on demand, removed from their sites of action by tissue uptake processes and metabolized mainly by fatty acid amide hydrolase (anandamide) or monoacylglycerol lipase (2-arachidonoyl glycerol).¹⁰ Endocannabinoids and their receptors constitute the 'endocannabinoid system'.

It is likely that endocannabinoids function as both neuromodulators and immunomodulators and indeed, there is already evidence that within the central nervous system they serve as retrograde synaptic messengers.¹¹ There is also evidence that there are some disease states or disorders in which endocannabinoids have an 'autoprotective' role. For example, results from animal experiments raise the possibility firstly, that increased amounts of endocannabinoid molecules may be released both in response to skeletal muscle spasm or spasticity in multiple sclerosis and in response to inflammatory pain, and secondly, that these released endocannabinoid molecules ameliorate such symptoms.^{8,12} Thus, in addition to CB₁ and CB₂ receptors, other important pharmacological targets within the endocannabinoid system are the processes responsible for the biosynthesis, membrane transport and metabolism of endocannabinoids, as modulators of these processes clearly have therapeutic potential.

Cannabinoid receptor agonists

There are several established cannabinoid receptor agonists that bind more or less equally well to

CB₁ and CB₂ receptors.¹ The best known examples of these are

- the 'classical' cannabinoids, Δ^9 -THC and (-)-11-hydroxy- Δ^8 -THC-dimethylheptyl (HU-210),
- the 'nonclassical' cannabinoid, CP55940,
- the aminoalkylindole cannabinoid, *R*-(+)-WIN55212, which has marginally greater CB₂ than CB₁ affinity, and
- the eicosanoid cannabinoids, anandamide, which has marginally greater CB₁ than CB₂ affinity, and 2-arachidonoyl glycerol.

Of these, HU-210 has the highest affinity for both CB₁ and CB₂ receptors and HU-210, CP55940, *R*-(+)-WIN55212 and 2-arachidonoyl glycerol have the highest CB₁ and CB₂ relative intrinsic activities. Δ^9 -THC and anandamide have lower CB₁ and CB₂ affinities and relative intrinsic activities than these other cannabinoids. Indeed, at both receptor types, Δ^9 -THC and anandamide each exhibit the mixed agonist-antagonist properties that are typical of a partial agonist.^{1,5} The recently discovered endocannabinoid, O-arachidonylethanolamine (virodhamine), is also a CB₁ receptor partial agonist and it too has been found to exhibit mixed agonist-antagonist properties at this receptor.¹³

A number of agonists with significant selectivity for CB₁ or CB₂ receptors have been developed.^{1,5} Important CB₁-selective agonists include the anandamide analogues, *R*-(+)-methanandamide, arachidonyl-2'-chloroethylamide (ACEA), arachidonyl-cyclopropylamide (ACPA) and O-1812. Of these both ACEA and ACPA share the susceptibility of anandamide to enzymic hydrolysis. In contrast, methanandamide and O-1812 are less susceptible to enzymic hydrolysis, probably because they are protected from this by the presence of a methyl substituent on the 1' carbon. Another CB₁-selective agonist of note is 2-arachidonoyl glyceryl ether (noladin ether). The best CB₂-selective agonists to have been developed so far include L-759633, L-759656 and JWH-133, all structural analogues of Δ^9 -THC, other notable examples being the nonclassical cannabinoid, HU-308, and the aminoalkylindole, AM1241.

Cannabinoid receptor agonists often contain chiral centres and these generally confer marked stereoselectivity in pharmacological assays. *R*-(+)-WIN55212 is more active than *S*-(-)-WIN55212 and classical and nonclassical cannabinoids with the same absolute stereochemistry as (-)- Δ^9 -THC at 6a and 10a (6aR, 10aR) have the greater activity. Anandamide itself does not contain any chiral centres. However, some of its synthetic analogues do, one example being methanandamide, the *R*-(+)-isomer of which has nine times greater affinity for CB₁ receptors than the *S*-(-)-isomer.¹

The discovery and pharmacological validation of new cannabinoid receptor agonists relies on the availability of suitable bioassays.^{1,5} For CB₁ receptor agonists, the most commonly used *in vivo* bioassay is the mouse tetrad, in which their ability to produce hypokinesia, hypothermia,

catalepsy in the Pertwee ring test and antinociception in the tail-flick or hot plate test is determined in the same animal. There are no standard *in vivo* bioassays for CB₂ receptor agonists. As to established *in vitro* bioassays for CB₁ and CB₂ receptor agonists, these all involve the use of membrane or tissue preparations that contain CB₁ and/or CB₂ receptors, expressed either naturally or after transfection.^{1,5,14} Among the most commonly used of these bioassays are binding assays that measure the ability of test compounds to displace a radiolabelled cannabinoid receptor ligand such as [³H]CP55940 from membranes obtained from CB₁ and/or CB₂ receptor-expressing cells or tissues. As to commonly used functional *in vitro* bioassays, some of these measure the effects of test compounds on CB₁ or CB₂ receptor signalling, for example stimulation of binding to G proteins of the hydrolysis-resistant GTP analogue [³⁵S]GTPγS, G_{i/o}-mediated inhibition of basal or drug-induced cyclic AMP production and elevation of intracellular free Ca²⁺, which is presumably a G_s-mediated effect. The bioassay of CB₁ receptor agonists can also be performed with isolated nerve-smooth muscle preparations such as the mouse vas deferens. These bioassays exploit the ability of cannabinoid agonists to act through neuronal CB₁ receptors to produce a concentration-related inhibition of electrically-evoked contractile transmitter release and hence of the contractions resulting from this release. Strategies commonly used to validate effects as CB₁ or CB₂ receptor-mediated rely on the availability of selective CB₁ and CB₂ receptor antagonists (Cannabinoid CB₁ and CB₂ receptor antagonists), of cells or tissues that express either CB₁ or CB₂ receptors (but not both these receptor types), or of animals or tissues from which CB₁ and/or CB₂ receptors have been genetically deleted.

Repeated administration of cannabinoid CB₁ receptor agonists can cause tolerance to develop to a number of their effects. This tolerance appears to be largely pharmacodynamic rather than pharmacokinetic in nature and to stem from CB₁ receptor internalization and/or from a reduction in CB₁ receptor protein synthesis or signalling.¹⁵ Similar mechanisms may underlie the development of tolerance to effects mediated by the CB₂ receptor.^{16,17}

Cannabinoid CB₁ and CB₂ receptor antagonists

The discovery of CB₁ and CB₂ receptors was followed by the development of CB₁- and CB₂-selective cannabinoid receptor antagonists.¹ Among these are the CB₁-selective SR141716A, AM251, AM281 and LY320135. These all produce inverse cannabimimetic effects in at least some CB₁ receptor-containing bioassay systems, effects that are opposite in direction from those produced by agonists for these receptors. Thus, for example, *in vivo* inverse effects of SR141716A in rats or mice include the production of signs of hyperalgesia in models of inflammatory and neuropathic pain, stimulation of intestinal motility and suppression of

food consumption, while its *in vitro* inverse effects include enhancement of ongoing release of acetylcholine, noradrenaline and γ-aminobutyric acid in hippocampal slices, enhancement of the amplitude of electrically-evoked contractions of the mouse isolated vas deferens and inhibition of [³⁵S]GTPγS binding to CB₁ receptors in membrane preparations.¹⁸

There is evidence that not all of the inverse cannabimimetic effects of SR141716A, AM251, AM281 or LY320135 are produced through a single mechanism.¹⁸ Thus, it is likely that some of these inverse effects result from competitive surmountable antagonism at CB₁ receptors of endogenously released endocannabinoids, a mechanism that is supported by evidence that such release takes place in a number of *in vivo* and *in vitro* bioassay systems. Some inverse cannabimimetic effects of ligands such as SR141716A are produced in the absence of any detectable ongoing endocannabinoid release. This is so both in systems into which CB₁ receptors have been genetically inserted, and so are usually over-expressed, and in systems in which these receptors are expressed naturally. It is likely that some of these inverse effects are induced by a process of 'inverse agonism' in which CB₁ receptors are shifted from a proposed constitutively active 'on' state to one or more constitutively inactive 'off' states. This putative mechanism relies on the assumption that CB₁ receptors can exist in a constitutively active state in which they undergo some degree of spontaneous coupling to their effector mechanisms even in the absence of an endogenously released or exogenously added agonist. Support for this mechanism comes from the findings that it has proved possible, firstly to devise a CB₁ mutant receptor at which SR141716A retains the ability to behave as an antagonist but loses its ability to produce an inverse cannabimimetic effect,¹⁹ and secondly to develop 'neutral' CB₁ receptor antagonists. These 'neutral' antagonists share the ability of SR141716A to block responses to CB₁ receptor agonists but lack its apparent ability to produce inverse cannabimimetic effects in CB₁-containing systems in the absence of any endogenously released or exogenously added CB₁ receptor agonist. Examples of 'neutral' CB₁ receptor antagonists are 6''-azidohept-2''-yne-cannabidiol (O-2654), O-2050, a sulphonamide analogue of Δ⁸-THC with an acetylenic sidechain, and two SR141716A analogues, VCHSR and NESS 0327.¹⁸ Although, by definition, a 'neutral' antagonist cannot change the degree of any constitutive activity exhibited by CB₁ receptors, it is expected to retain the ability to produce inverse cannabimimetic effects in tonically active biological systems when this tonic activity arises from ongoing endocannabinoid release onto CB₁ receptors.

Not all inverse cannabimimetic effects of CB₁ receptor ligands seem to be induced through CB₁ receptor-dependent mechanisms. Thus, for example, there is evidence that at 10 μM, SR141716A and AM251 inhibit [³⁵S]GTPγS binding to rat cerebellar membranes by blocking the activation of A₁ receptors by endogenously released adenosine.²⁰ It has also been reported that at concentrations greater than 1 μM

SR141716A has a number of other actions that include inhibition of basal [35 S]GTP γ S binding to membranes obtained from CB $_1^{-/-}$ mice and blockade of gap junctions and of certain types of ion channel.^{18,21}

SR141716A appears to be more potent at opposing effects induced by CB $_1$ agonists than at producing inverse cannabimimetic effects by itself either at CB $_1$ receptors or through CB $_1$ receptor-independent mechanisms.¹⁸ It is possible, therefore, that there may be a low concentration range within which this ligand is essentially a neutral antagonist and that it is only at higher concentrations that it exhibits inverse agonist properties.

As to CB $_2$ -selective antagonists, the best known of these are SR144528 and AM630, both of which also produce inverse cannabimimetic effects in at least some cannabinoid receptor-containing bioassay systems.¹ However, the mechanisms underlying the production of inverse effects by these two compounds have been little investigated.

The therapeutic potential of cannabinoid CB $_1$ and CB $_2$ receptor ligands

Dronabinol (Marinol), an oral preparation of Δ^9 -THC, and nabilone, a synthetic analogue of Δ^9 -THC are already licenced for clinical use in some countries as appetite stimulants (dronabinol) and antiemetics (both drugs). For CB $_1$ receptor agonists, other potential uses include the management of glaucoma, pain, certain types of cancer and various kinds of motor dysfunction associated for example with multiple sclerosis or spinal cord injury.^{8,12,22,23} Particularly convincing are preclinical, anecdotal and clinical data supporting the use of CB $_1$ receptor agonists against inflammatory and neuropathic pain and for the amelioration of spasticity, muscle spasms, tremor or pain associated with multiple sclerosis or spinal cord injury.^{8,12} Indeed, a Δ^9 -THC-containing extract of cannabis has now been developed in the UK as a medicine for the management of some multiple sclerosis symptoms. As to CB $_2$ receptor agonists, there is already strong evidence that these have potential for the relief of inflammatory pain and, unexpectedly, also for the relief of neuropathic pain.²⁴ While therapeutic targets for CB $_2$ receptor inverse agonists/antagonists remain to be identified or validated, it is likely that the CB $_1$ receptor inverse agonist/antagonist, SR141716A (Rimonabant/Acomplia), will soon be available in the clinic as an antiobesity agent.^{25,26}

Other pharmacological targets for cannabinoid receptor ligands

It is now clear that the TRPV1 (vanilloid VR1) receptor can be activated by anandamide, methanandamide and arachidonyl-2'-chloroethylamide (ACEA), although not by 2-arachi-

donoyl glycerol or by classical, nonclassical or aminoalkylindole cannabinoid receptor agonists (Cannabinoid receptor agonists) such as HU-210, CP55940 and R-(+)-WIN55212.^{5,9,27,28} Evidence is also emerging for the existence of several other pharmacological targets that respond to at least some established cannabinoid receptor agonists or to abnormal-cannabidiol, a synthetic analogue of the plant cannabinoid cannabidiol that lacks significant affinity for both CB $_1$ and CB $_2$ receptors.^{5,9} These include:

- TRPV1-like receptors that are activated by WIN55212-2, CP55940 and capsaicin and that mediate inhibition of release of the excitatory neurotransmitter, glutamate, in brain areas such as the hippocampus;
- various non-CB $_1$, non-CB $_2$, non-TRPV1 targets on central or peripheral neurones that modulate transmitter release when activated;
- a non-neuronal target in mesenteric arteries that can be activated by abnormal-cannabidiol, methanandamide and anandamide but not Δ^9 -THC, HU-210, WIN55212-2 or 2-arachidonoyl glycerol to trigger reversal of α_1 -adrenoceptor-mediated vasoconstriction;
- a target for abnormal-cannabidiol on microglial cells that can be activated to trigger migration of these cells towards neuroinflammatory lesion sites;
- an SR144528-sensitive, SR141716A-insensitive, anandamide-insensitive, non-TRPV1, 'CB $_2$ -like' peripheral target through which palmitoylethanolamide, which lacks significant affinity for CB $_1$ or CB $_2$ receptors, can relieve inflammatory pain;
- an allosteric site on the 5-HT $_3$ receptor at which Δ^9 -THC can inhibit inward current through this ligand-gated cation channel with greater potency than cannabinoids such as R-(+)-WIN55212, anandamide, LY320135 and CP55940;
- allosteric sites on GLU A_1 and GLU A_3 receptors, on M $_1$ and M $_4$ muscarinic receptors and on delayed rectifier potassium channels.

There is also evidence firstly, that palmitoylethanolamide can augment anandamide-induced microglial cell migration by acting through G $_{i/o}$ -coupled receptors that are not CB $_1$, CB $_2$, 'CB $_2$ -like' or abnormal-cannabidiol receptors and secondly, that cannabinoids such as Δ^9 -THC and cannabidiol that contain a phenol group possess antioxidant (electron donor) activity that is sufficient to protect neurones against oxidative stress associated, for example, with glutamate-induced excitotoxicity.⁵

CB $_1$ receptor antagonists/inverse agonists also appear to have pharmacological targets in addition to the CB $_1$ receptor. For example, at concentrations above those at which it is capable of blocking the CB $_1$ receptor, SR141716A behaves as an antagonist of the CB $_2$ receptor, the putative abnormal-cannabidiol receptor, the adenosine A $_1$ receptor, the TRPV1 receptor and possibly also the TRPV1-like receptor.^{18,20}

Finally, evidence is now emerging for the presence of an allosteric site on the CB₁ receptor.²⁹ Thus, we have found a series of novel compounds to behave as allosteric CB₁ receptor modulators. These compounds do not displace [³H]CP55940 from CB₁ binding sites but do modulate the rate at which [³H]CP55940 dissociates from these sites. This discovery opens up the possibility of developing allosteric CB₁ antagonists instead of competitive CB₁ receptor antagonists for the clinic (e.g. as antiobesity agents) and of developing allosteric CB₁ enhancers that might be used therapeutically to augment the effects of endocannabinoids when these are released autoprotectively, for example in inflammatory pain conditions or in multiple sclerosis (Endogenous agonists for cannabinoid receptors).

Cannabinoid receptor research: future directions

A number of issues remain to be resolved. In particular, it will be important to establish

- the extent to which proposed non-CB₁, non-CB₂ targets for CB₁ or CB₂ receptor agonists and antagonists/inverse agonists contribute to the pharmacology of these ligands, especially at clinically-relevant concentrations, or mediate physiological or pathological events of clinical importance;
- whether it will prove possible to develop potent and selective non-CB₁, non-CB₂ agonists and antagonists for each of these proposed new targets;
- the extent to which allosteric antagonism of the 5-HT₃ receptor by Δ^9 -THC contributes towards the well-established antiemetic activity of this cannabinoid;
- the mechanism by which CB₂ receptors alleviate neuropathic pain if, as currently believed, CB₂ receptors are not expressed by neurones;
- whether the likely presence of an allosteric site on the CB₁ receptor can be exploited in the clinic, for example, by employing an allosteric inhibitor as an anti-obesity agent or by using an allosteric enhancer for the management multiple sclerosis or chronic pain;
- the extent to which cannabinoid receptors form CB₁-CB₁ and CB₂-CB₂ homodimers;
- whether cannabinoid receptors form heterodimers or oligomers and, if so, the nature and extent of the resulting cross-talk between the endocannabinoid system and other endogenous systems.

Three other important issues that merit further investigation relate to the proposed 'autoprotective' role of endocannabinoids in some disease states (Endogenous agonists for cannabinoid receptors). The first of these concerns CB₁ receptor antagonists/inverse agonists and neutral antagonists when these are used in the clinic, for example as appetite suppressants. Thus, the possibility arises that these drugs will enhance unwanted symptoms such as spasm,

spasticity and pain if given to patients with multiple sclerosis or with disorders that cause inflammatory cause inflammatory pain in whom autoprotective release of endocannabinoids onto CB₁ receptors is taking place.

The second of these issues concerns the potential use in the clinic of inhibitors of endocannabinoid membrane transport or of endocannabinoid enzymic hydrolysis by fatty acid amide hydrolase or monoacylglycerol lipase. Such inhibitors are likely to have fewer CB₁ receptor-mediated side effects than directly-acting CB₁ agonists as they are expected to augment CB₁ receptor activation only in those parts of the endocannabinoid system in which endocannabinoid release is taking place. However, this does not necessarily mean that they will prove to have fewer or less marked unwanted side effects than directly-acting CB₁ receptor agonists. For example, they may produce a new set of CB₁ receptor-independent unwanted effects by inhibiting the enzymic inactivation of pharmacologically active endogenous molecules that do not serve as endocannabinoids or by causing an accumulation of endocannabinoid molecules at non-CB₁, non-CB₂ targets such as the TRPV1 receptor or the putative abnormal-cannabidiol receptor.^{5,9} It is also to be expected that a fatty acid amide hydrolase inhibitor will change the pattern of anandamide metabolism so that there is a greater conversion of anandamide to pharmacologically active cyclooxygenase and/or lipoxygenase metabolites.¹¹ The formation of cyclooxygenase metabolites will be even greater if there has also been induction of cyclooxygenase-2 by an inflammatory stimulus.

The third issue is whether CB₁ receptor allosteric enhancers can be developed for the clinic. If they can, it is likely that they will prove to be even more selective than inhibitors of endocannabinoid membrane transport or enzymic hydrolysis in augmenting endocannabinoid-induced autoprotection to therapeutic advantage. Thus, like the transport and enzyme inhibitors, they are expected to increase CB₁ receptor activation only at sites at which endocannabinoid release is taking place, thereby giving rise to fewer unwanted effects than directly-acting CB₁ receptor agonists. However, unlike endocannabinoid transport and enzyme inhibitors they are not expected to augment the activation of non-CB₁ targets by endocannabinoids, by endocannabinoid metabolites or by pharmacologically-active non-endocannabinoid substrates of endocannabinoid transporters or metabolizing enzymes.

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