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REVIEW

The pharmacology of cannabinoid receptors and their ligands: an overview

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Mammalian tissues express at least two cannabinoid receptor types, CB_1 and CB_2, both G protein coupled. CB_1 receptors are found predominantly at nerve terminals where they mediate inhibition of transmitter release. CB_2 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 arachidonoylethanolamide 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-CB_1, non-CB_2, non-TRPV1 targets. Several CB_1- and CB_2-selective agonists and antagonists have been developed. Antagonists include the CB_1-selective SR141716A, AM251, AM281 and LY320135, and the CB_2-selective SR144528 and AM630. These all behave as inverse agonists, one indication that CB_1 and CB_2 receptors can exist in a constitutively active state. 'Neutral' cannabinoid receptor antagonists have also been developed. CB_1 and/or CB_2 receptor activation appears to ameliorate inflammatory and neuropathic pain and certain multiple sclerosis symptoms. This might be exploited clinically by using CB_1, CB_2 or CB_1/CB_2 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 CB_1 receptor. Consequently, it may also prove possible to enhance 'autoprotective' effects of released endocannabinoids with CB_1 allosteric enhancers or, indeed, to reduce proposed 'autoimpairing' effects of released endocannabinoids such as excessive food intake with CB_1 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 CB_1 receptors, cloned in Tom Bonner's laboratory in 1990,[2] and CB_2 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 CB_1 and CB_2 receptors are coupled through $G_{i/o}$ proteins, negatively to adenylate cyclase and positively to mitogen-activated protein kinase. CB_1 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 channels[4] and to N-type and P/Q type calcium channels. CB_1 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 CB_1 and CB_2 receptors can be found elsewhere.[1]

Although CB_1 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] CB_2 receptors occur mainly on immune cells, one of their roles being to modulate cytokine release. Thus, a common role of CB_1 and CB_2 receptors appears to be the modulation of ongoing release of chemical messengers, CB_2 receptors from immune cells and CB_1 receptors mainly from neurones.[1] Interestingly, evidence is emerging that CB_1 receptors can exist as homodimers and also that they may form

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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 CB_1 and non-CB_1 receptors.[5] Indeed, it has been proposed that on-going cross-talk between presynaptic CB_1 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 CB_1 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, CB_2 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 arachidonoylethanolamide (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 CB_1 and CB_2 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 CB_1 and CB_2 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 CB_2 than CB_1 affinity, and
- the eicosanoid cannabinoids, anandamide, which has marginally greater CB_1 than CB_2 affinity, and 2-arachidonoyl glycerol.

Of these, HU-210 has the highest affinity for both CB_1 and CB_2 receptors and HU-210, CP55940, *R*-(+)-WIN55212 and 2-arachidonoyl glycerol have the highest CB_1 and CB_2 relative intrinsic activities. Δ^9-THC and anandamide have lower CB_1 and CB_2 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*-arachidonoylethanolamine (virodhamine), is also a CB_1 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 CB_1 or CB_2 receptors have been developed.[1,5] Important CB_1-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 CB_1-selective agonist of note is 2-arachidonyl glyceryl ether (noladin ether). The best CB_2-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 (6a*R*, 10a*R*) 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 CB_1 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 CB_1 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 CB_2 receptor agonists. As to established *in vitro* bioassays for CB_1 and CB_2 receptor agonists, these all involve the use of membrane or tissue preparations that contain CB_1 and/or CB_2 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 [^{3}H]CP55940 from membranes obtained from CB_1 and/or CB_2 receptor-expressing cells or tissues. As to commonly used functional *in vitro* bioassays, some of these measure the effects of test compounds on CB_1 or CB_2 receptor signalling, for example stimulation of binding to G proteins of the hydrolysis-resistant GTP analogue [^{35}S]GTPγS, $G_{i/o}$-mediated inhibition of basal or drug-induced cyclic AMP production and elevation of intracellular free Ca^{2+}, which is presumably a G_s-mediated effect. The bioassay of CB_1 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 CB_1 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 CB_1 or CB_2 receptor-mediated rely on the availability of selective CB_1 and CB_2 receptor antagonists (Cannabinoid CB_1 and CB_2 receptor antagonists), of cells or tissues that express either CB_1 or CB_2 receptors (but not both these receptor types), or of animals or tissues from which CB_1 and/or CB_2 receptors have been genetically deleted.

Repeated administration of cannabinoid CB_1 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 CB_1 receptor internalization and/or from a reduction in CB_1 receptor protein synthesis or signalling.[15] Similar mechanisms may underlie the development of tolerance to effects mediated by the CB_2 receptor.[16,17]

Cannabinoid CB_1 and CB_2 receptor antagonists

The discovery of CB_1 and CB_2 receptors was followed by the development of CB_1- and CB_2-selective cannabinoid receptor antagonists.[1] Among these are the CB_1-selective SR141716A, AM251, AM281 and LY320135. These all produce inverse cannabimimetic effects in at least some CB_1 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 [^{35}S]GTPγS binding to CB_1 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 CB_1 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 CB_1 receptors have been genetically inserted, and so are usually overexpressed, 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 CB_1 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 CB_1 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 CB_1 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’ CB_1 receptor antagonists. These ‘neutral’ antagonists share the ability of SR141716A to block responses to CB_1 receptor agonists but lack its apparent ability to produce inverse cannabimimetic effects in CB_1-containing systems in the absence of any endogenously released or exogenously added CB_1 receptor agonist. Examples of ‘neutral’ CB_1 receptor antagonists are 6″-azidohex-2″-yne-cannabidiol (O-2654), O-2050, a sulphonamide analogue of Δ^8-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 CB_1 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 CB_1 receptors.

Not all inverse cannabimimetic effects of CB_1 receptor ligands seem to be induced through CB_1 receptor-dependent mechanisms. Thus, for example, there is evidence that at 10 μM, SR141716A and AM251 inhibit [^{35}S]GTPγS binding to rat cerebellar membranes by blocking the activation of A_1 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]GTPγS 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-arachidonoyl 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_{A1} and GLU_{A3} 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 CB_1 receptor.[29] Thus, we have found a series of novel compounds to behave as allosteric CB_1 receptor modulators. These compounds do not displace [^{3}H]CP55940 from CB_1 binding sites but do modulate the rate at which [^{3}H]CP55940 dissociates from these sites. This discovery opens up the possibility of developing allosteric CB_1 antagonists instead of competitive CB_1 receptor antagonists for the clinic (e.g. as antiobesity agents) and of developing allosteric CB_1 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-CB_1, non-CB_2 targets for CB_1 or CB_2 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-CB_1, non-CB_2 agonists and antagonists for each of these proposed new targets;
- the extent to which allosteric antagonism of the 5-HT_3 receptor by Δ^9-THC contributes towards the well-established antiemetic activity of this cannabinoid;
- the mechanism by which CB_2 receptors alleviate neuropathic pain if, as currently believed, CB_2 receptors are not expressed by neurones;
- whether the likely presence of an allosteric site on the CB_1 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 CB_1-CB_1 and CB_2-CB_2 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 CB_1 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 CB_1 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 CB_1 receptor-mediated side effects than directly-acting CB_1 agonists as they are expected to augment CB_1 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 CB_1 receptor agonists. For example, they may produce a new set of CB_1 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-CB_1, non-CB_2 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 CB_1 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 CB_1 receptor activation only at sites at which endocannabinoid release is taking place, thereby giving rise to fewer unwanted effects than directly-acting CB_1 receptor agonists. However, unlike endocannabinoid transport and enzyme inhibitors they are not expected to augment the activation of non-CB_1 targets by endocannabinoids, by endocannabinoid metabolites or by pharmacologically-active non-endocannabinoid substrates of endocannabinoid transporters or metabolizing enzymes.

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