

Pharmacological Actions of Cannabinoids

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Abstract Mammalian tissues express at least two types of cannabinoid receptor, CB₁ and CB₂, both G protein coupled. CB₁ receptors are expressed predominantly at nerve terminals where they mediate inhibition of transmitter release. CB₂ receptors

are found mainly on immune cells, one of their roles being to modulate cytokine release. Endogenous ligands for these receptors (endocannabinoids) also exist. These are all eicosanoids; prominent examples include arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol. These discoveries have led to the development of CB₁- and CB₂-selective agonists and antagonists and of bioassays for characterizing such ligands. Cannabinoid receptor 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 that seem to lack inverse agonist properties have recently also been developed. As well as acting on CB₁ and CB₂ receptors, there is convincing evidence that anandamide can activate transient receptor potential vanilloid type 1 (TRPV1) receptors. Certain cannabinoids also appear to have non-CB₁, non-CB₂, non-TRPV1 targets, for example CB₂-like receptors that can mediate antinociception and “abnormal-cannabidiol” receptors that mediate vasorelaxation and promote microglial cell migration. There is evidence too for TRPV1-like receptors on glutamatergic neurons, for α_2 -adrenoceptor-like (imidazoline) receptors at sympathetic nerve terminals, for novel G protein-coupled receptors for R-(+)-WIN55212 and anandamide in the brain and spinal cord, for novel receptors for Δ^9 -tetrahydrocannabinol and cannabidiol on perivascular sensory nerves and for novel anandamide receptors in the gastro-intestinal tract. The presence of allosteric sites for cannabinoids on various ion channels and non-cannabinoid receptors has also been proposed. In addition, more information is beginning to emerge about the pharmacological actions of the non-psychoactive plant cannabinoid, cannabidiol. These recent advances in cannabinoid pharmacology are all discussed in this review.

Keywords Cannabinoid receptors · Cannabinoid receptor agonists and antagonists · Abnormal-cannabidiol · Cannabidiol · Inverse agonism

1

Introduction

“Cannabinoid” was originally the collective name given to a set of oxygen-containing C₂₁ aromatic hydrocarbon compounds that occur naturally in the plant *Cannabis sativa* (ElSohly 2002; Mechoulam and Gaoni 1967). However, this term is now generally also used for all naturally occurring or synthetic compounds that can mimic the actions of plant-derived cannabinoids or that have structures that closely resemble those of plant cannabinoids. Consequently, a separate term, “phyto-cannabinoid”, has been coined for the cannabinoids produced by cannabis (Pate 1999). One phytocannabinoid, Δ^9 -tetrahydrocannabinol (Δ^9 -THC; Fig. 1), has attracted particular attention. This is because it is the main psychoactive constituent of cannabis (reviewed in Pertwee 1988) and because it is one of just two cannabinoids to be licensed for medical use, the other being nabilone (Cesamet; Fig. 2), a synthetic analogue of Δ^9 -THC (reviewed in the chapter by Robson, this vol-

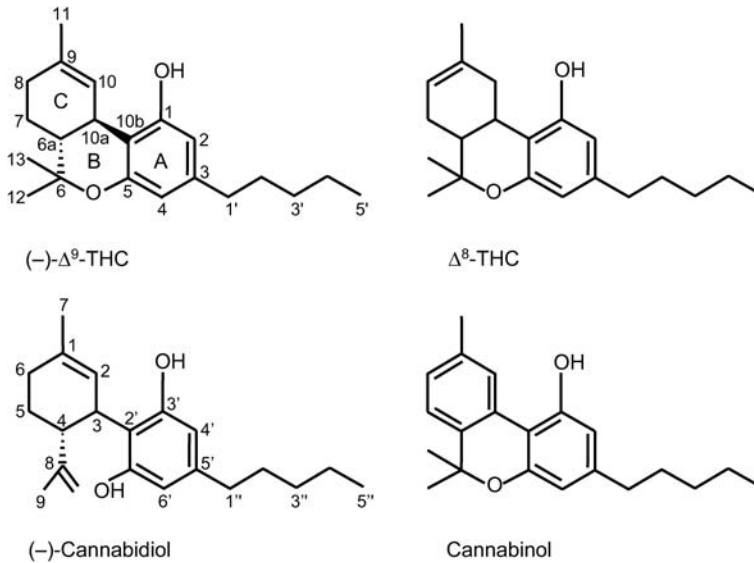


Fig. 1. The structures of four plant cannabinoids, Δ^9 -THC, Δ^8 -THC, cannabinol and cannabidiol

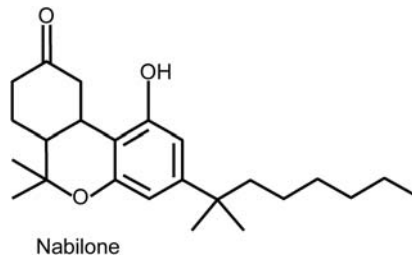


Fig. 2. The structure of nabilone

ume). Because of its high lipid solubility and low water solubility, Δ^9 -THC was long thought to owe its pharmacological properties to an ability to perturb the phospholipid constituents of biological membranes (reviewed in Pertwee 1988). However, all this changed in the late 1980s with the discovery in mammalian tissues of specific cannabinoid receptors.

Two types of cannabinoid receptor have so far been identified (reviewed in Howlett et al. 2002). These are CB₁, cloned in Tom Bonner's laboratory in the USA in 1990, and CB₂, cloned by Sean Munro in the UK in 1993. Both these 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, positively to A-type and inwardly rectifying potassium channels and negatively to N-type and P/Q-type calcium channels and to D-type potassium channels. In addition, there are reports that CB₁ and CB₂ receptors can enhance intracellular free Ca²⁺ concentrations (Fan and Yazulla 2003; Rubovitch et al. 2002; Sugiura et al. 1996, 1997, 2000). It is unclear whether this enhancement is G_{i/o}

mediated. In experiments with NG108-15 cells, Sugiura et al. (1996) found CB₁-mediated increases in intracellular free Ca²⁺ levels to be abolished by pretreatment with pertussis toxin, pointing to an involvement of G_{i/o} proteins. However, in experiments with N18TG2 neuroblastoma cells, Rubovich et al. (2002) reported that pertussis toxin failed to prevent CB₁-mediated enhancement of intracellular free Ca²⁺ levels by low concentrations of desacetyl-L-nantradol, a cannabinoid receptor agonist (Sect. 3.1), and instead unmasked a stimulatory effect of higher concentrations of this agonist that in the absence of pertussis toxin did not alter intracellular free Ca²⁺ levels at all. Rubovich et al. (2002) also obtained evidence that the stimulatory effect of desacetyl-L-nantradol on intracellular Ca²⁺ release depended on an ability to delay the inactivation of open L-type voltage-dependent calcium channels and that it was mediated mainly by cyclic AMP-dependent protein kinase (PKA).

Although there is no doubt that G_{i/o} proteins play a major role in cannabinoid receptor signalling, there is also no doubt that transfected and naturally expressed CB₁ receptors can act through G_s proteins to activate adenylate cyclase (Calandra et al. 1999; Glass and Felder 1997; Maneuf and Brotchie 1997). The extent to which CB₁ receptors signal through G_s proteins may be determined by CB₁ receptor location or by cross-talk with colocalized G protein-coupled non-CB₁ receptors (Breivogel and Childers 2000; Calandra et al. 1999; Glass and Felder 1997; Jarraghan et al. 2004). As proposed by Calandra et al. (1999), it is also possible that there are distinct subpopulations CB₁ receptors, one coupled to G_{i/o} proteins and the other to G_s. Additional signalling mechanisms for cannabinoid CB₁ and CB₂ receptors have been proposed and descriptions of these can be found elsewhere (Howlett et al. 2002; see also the chapter by Howlett, this volume).

CB₁ receptors are expressed by central and peripheral neurons and also by some nonneuronal cells (reviewed in Howlett et al. 2002; Pertwee 1997; see also the chapter by Mackie, this volume). Within the central nervous system, the distribution pattern of CB₁ receptors is heterogeneous and can account for several of the characteristic pharmacological properties of CB₁ receptor agonists. For example, the presence of large populations of CB₁ receptors in cerebral cortex, hippocampus, caudate-putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus and cerebellum, as well as in some areas of the brain and spinal cord that process or modulate nociceptive information, probably accounts for the ability of CB₁ receptor agonists to impair cognition and memory, to alter the control of motor function and to produce antinociception (reviewed in Iversen 2003; Pertwee 2001; see also the chapters by Riedel and Davies, Fernández-Ruiz and González, and Walker and Hohmann, this volume). Some CB₁ receptors are located at central and peripheral nerve terminals. Here they modulate the release of excitatory and inhibitory neurotransmitters when activated (Howlett et al. 2002). Although the effect of CB₁ receptor agonists on release that has been most often observed is one of inhibition, there has been one report that the CB₁/CB₂ receptor agonist, *R*-(+)-WIN55212 (Sect. 3.1), can act through CB₁ receptors to stimulate release of glutamate from primary cultures of rat cerebral cortical neurons (Ferraro et al. 2001). This effect, which disappeared when the concentration of *R*-(+)-WIN55212 was increased from 1 or 10 nM to 100 nM, was most probably triggered by cal-

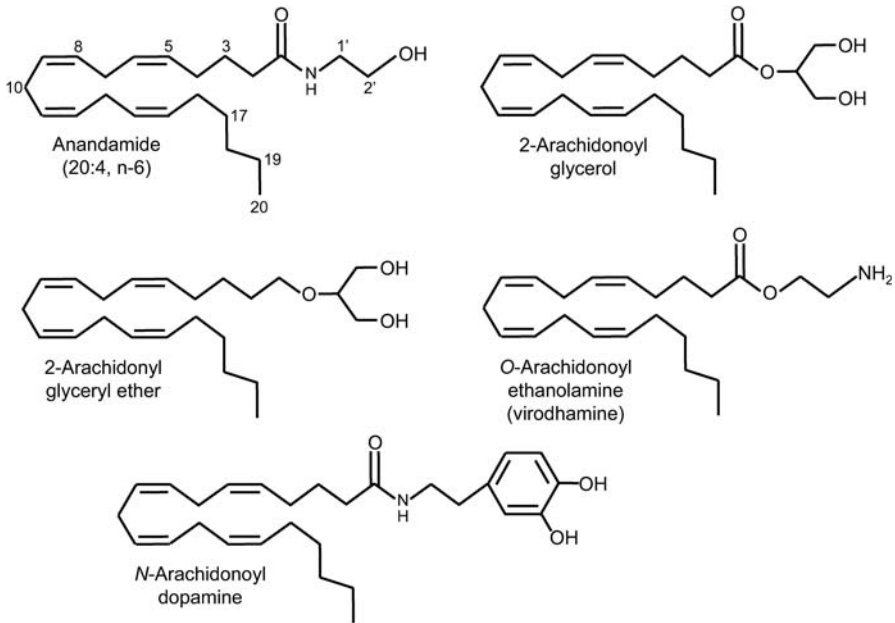


Fig. 3. The structures of five putative endogenous cannabinoids

cium released from inositol 1,4,5-triphosphate-controlled intracellular stores in response to a CB₁ receptor-mediated activation of phospholipase C. CB₂ receptors are expressed mainly by immune cells that include lymphocytes, macrophages, mast cells, natural killer cells, peripheral mononuclear cells and microglia (reviewed in Howlett et al. 2002; Pertwee 1997; see also the chapter by Cabral and Staab, this volume). Less is known about the roles of CB₂ than of CB₁ receptors, although there is good evidence that CB₂ receptors can trigger microglial cell migration (Sect. 4.1.5) and regulate cytokine release. Thus, one property CB₁ and CB₂ receptors share is the ability to modulate ongoing release of chemical messengers.

The discovery of cannabinoid receptors was followed by the demonstration that mammalian tissues can produce endogenous agonists for these receptors, all of which have so far proved to be derivatives of arachidonic acid (reviewed in Di Marzo et al. 1998; Hillard 2000; Mechoulam et al. 1998; see also the chapter by Di Marzo et al., this volume). The most investigated of these “endocannabinoids” have been arachidonylethanolamine (anandamide) and 2-arachidonoyl glycerol (Fig. 3), both of which are synthesized on demand rather than stored. Other compounds that may be endocannabinoids include 2-arachidonylglyceryl ether (noladin ether), O-arachidonylethanolamine (virodhamine) and N-arachidonoyldopamine (Howlett et al. 2002; Porter et al. 2002; Walker et al. 2002). Endocannabinoids together with cannabinoid receptors constitute what is now usually referred to as 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

(reviewed in the chapter by Vaughan and Christie, this volume). There is also evidence that following their release, anandamide and 2-arachidonoyl glycerol enter cells by a combination of simple diffusion and facilitated, carrier-mediated transport (reviewed in Hillard and Jarrahian 2003) and are then metabolized by intracellular enzymes, anandamide by fatty acid amide hydrolase and 2-arachidonoyl glycerol mainly by monoacylglycerol lipase (monoglyceride lipase) but also by fatty acid amide hydrolase (reviewed in Cravatt and Lichtman 2002; Dinh et al. 2002; Ueda 2002; van der Stelt and Di Marzo 2004; see also the chapter by Di Marzo et al., this volume). Noladin ether also seems to be a substrate for anandamide/2-arachidonoyl glycerol membrane transporter(s) (Fezza et al. 2002). The processes responsible for the production, membrane transport and enzymic inactivation of endocannabinoids are all pharmacological targets through which the activity of the endocannabinoid system can or might be modulated to experimental or therapeutic advantage (reviewed in the chapters by Howlett and by Di Marzo et al., this volume). There is evidence that such modulation may also take place naturally as a result of the co-release of endogenous fatty acid derivatives such as palmitoylethanolamide and oleamide, which can potentiate anandamide, or of 2-linoleyl glycerol and 2-palmitoyl glycerol, which can potentiate 2-arachidonoyl glycerol (Mechoulam et al. 1998). For anandamide, mechanisms through which co-released ligands induce this “entourage effect” include not only inhibition of its metabolism by fatty acid amide hydrolase but also increases in the sensitivity of CB₁ or vanilloid receptors or of other pharmacological targets for anandamide through allosteric or other mechanisms (De Petrocellis et al. 2001b, 2002; Franklin et al. 2003; Mechoulam et al. 1998; Smart et al. 2002).

This chapter describes the *in vitro* and *in vivo* bioassays that have been most widely used to characterize ligands for CB₁ and/or CB₂ receptors and reviews the ability of compounds commonly used in cannabinoid research as experimental tools to activate or block these receptors. The likelihood that the most widely used cannabinoid receptor antagonists are inverse agonists rather than neutral antagonists is also discussed, as is evidence for the presence in mammalian tissues of non-CB₁, non-CB₂ pharmacological targets for cannabinoids.

2 **Bioassays for Characterizing CB₁ and CB₂ Receptor Ligands**

2.1 **In Vitro Binding Assays**

Several cannabinoid receptor ligands have been radiolabelled with tritium, and these have been used both to determine the CB₁ and CB₂ receptor affinities of unlabelled cannabinoids in displacement assays and to establish the tissue distribution patterns of these receptors (reviewed in Howlett et al. 2002; Pertwee 1999a). As indicated in Tables 1, 2 and 3, some of these compounds bind more readily to CB₁ or to CB₂ receptors, whilst the others bind more or less equally well to both these

Table 1. Typical dissociation constant (K_D) values of radiolabelled ligands at cannabinoid receptor CB₁ and CB₂ binding sites

Radioligand	Source of membranes	Receptor	K_D (nM)	Reference(s)
³ H]SR141716A	Rat brain ^a	rCB ₁	0.19–1.20	For references, see Pertwee 1999a
	Guinea-pig forebrain	g-pCB ₁	1.24	
[¹²³ I]AM251	Rat cerebellum	rCB ₁	0.25	
³ H]R-(+)-WIN55212	Rat cerebellum	rCB ₁	1.89, 4.67, 8.6	
	Guinea-pig forebrain	g-pCB ₁	2.34	
	Cultured cells ^b	rCB ₁	2.60	
	Cultured cells ^b	hCB ₁	16.2, 11.9	
	Cultured cells ^b	hCB ₂	3.7, 3.8	
³ H]HU-210 (HU-243)	Rat brain minus brain stem	rCB ₁	0.045	
	Cultured cells ^b	hCB ₂	0.061	
³ H]CP55940	Cultured cells ^b	hCB ₁	0.4 to 3.3	For references, see Pertwee 1997, 1999a
	Cultured cells ^b	rCB ₁	4	
	Rat brain ^a	rCB ₁	0.07 to 2.3	
	Mouse whole brain	mCB ₁	3.4	
	Cultured cells ^b	hCB ₂	0.2 to 7.4	
³ H]CP55940	Cultured cells ^b	mCB ₂	0.39	Mauler et al. 2002
	Rat cerebellum	rCB ₁	2.37	
	Human cerebral cortex	hCB ₁	1.29	
	Cultured cells ^b	hCB ₁	1.10	
³ H]BAY 38-7271	Cultured cells ^b	hCB ₂	4.20	Mauler et al. 2002
	Rat cerebellum	rCB ₁	1.84	
	Human cerebral cortex	hCB ₁	2.10	
	Cultured cells ^b	hCB ₁	2.91	
	Cultured cells ^b	hCB ₂	4.24	

g-pCB₁, Guinea-pig CB₁ receptors; hCB₁ and hCB₂, human cannabinoid receptors; mCB₁ and mCB₂, mouse cannabinoid receptors; rCB₁ and rCB₂, rat cannabinoid receptors.

^aWhole brain or a discrete area.

^bCells transfected with CB₁ or CB₂ receptors.

receptor types. It is noteworthy, therefore, that CB₁ or CB₂ selectivity can still be achieved in displacement assays with the non-selective radiolabelled ligands by using membranes obtained from cannabinoid receptor-free cultured cells that have been transfected with CB₁ or CB₂ receptors or membranes obtained from brain (CB₁-rich) or spleen (CB₂-rich). Some care is needed in interpreting binding data obtained with brain or spleen membranes. Thus, whilst there is little evidence that CB₂ receptors are expressed by central neurons, these receptors are expressed by microglial cells (Howlett et al. 2002). Similarly, although it is mainly CB₂ receptors that are present in spleen, this tissue also expresses some CB₁ receptors (reviewed in Howlett et al. 2002; Pertwee 1997). Moreover, there is growing evidence for the presence in brain and other tissues of non-CB₁, non-CB₂ cannabinoid recep-

Table 2. Examples of K_i values of certain cannabinoid CB₁ and/or CB₂ receptor agonists for the in vitro displacement of [³H]CP55940, [³H]HU243 or [³H]BAY-38-7271 from CB₁- and CB₂-specific binding sites (continued on next page)

Agonist	CB ₁ K_i value (nM)	CB ₂ K_i value (nM)	Reference
CB₁-selective agonists in order of decreasing CB₁/CB₂ selectivity			
ACEA	1.4 ^{a,b}	>2,000 ^{a,b}	Hillard et al. 1999
	5.29 ^{a,b}	195 ^c	Lin et al. 1998
O-1812	3.4 ^b	3,870 ^b	Di Marzo et al. 2001
ACPA	2.2 ^{a,b}	715 ^{a,b}	Hillard et al. 1999
2-Arachidonyl glyceryl ether	21.2 ^b	>3,000 ^d	Hanus et al. 2001
<i>R</i> -(+)-methanandamide	17.9 ^{a,b}	868 ^c	Lin et al. 1998
	20 ^{a,b}	815 ^c	Khanolkar et al. 1996
	28.3 ^b	868 ^c	Goutopoulos et al. 2001
Agonists without any marked CB₁ or CB₂ selectivity			
Anandamide	61 ^{a,b}	1,930 ^c	Lin et al. 1998
	78.2 ^{a,b}	1,926 ^c	Khanolkar et al. 1996
	89 ^a	371 ^a	Showalter et al. 1996
	543	1,940	Felder et al. 1995
	71.7 ^{a,b}	279 ^{a,b}	Hillard et al. 1999
	252 ^{e,d}	581 ^{e,d}	Mechoulam et al. 1995
BAY 38-7271	1.85 ^f	5.96 ^f	Mauler et al. 2002
2-Arachidonoyl glycerol	472 ^{e,d}	1,400 ^{e,d}	Mechoulam et al. 1995
	58.3 ^{e,d}	145 ^{e,d}	Ben-Shabat et al. 2000
O-1057	4.4	11.2	Pertwee et al. 2000
HU-210	0.0608	0.524	Felder et al. 1995
	0.1 ^{e,b}	0.17 ^e	Rhee et al. 1997
	0.73	0.22	Showalter et al. 1996
CP55940	5	1.8	Ross et al. 1999a
	3.72	2.55	Felder et al. 1995
	1.37 ^b	1.37 ^b	Rinaldi-Carmona et al. 1994
	0.58	0.69	Showalter et al. 1996
	0.50 ^{a,b}	2.80 ^{a,b}	Hillard et al. 1999
Δ^9 -THC	53.3	75.3	Felder et al. 1995
	39.5 ^{e,b}	40 ^e	Bayewitch et al. 1996
	40.7	36.4	Showalter et al. 1996
	80.3 ^{e,b}	32.2 ^e	Rhee et al. 1997
	35.3 ^b	3.9 ^b	Rinaldi-Carmona et al. 1994
Nabilone	5.05	3.13	Iwamura et al. 2001
	1.84	2.19	Gareau et al. 1996
Δ^8 -THC	47.6 ^b	39.3 ^c	Busch-Petersen et al. 1996
	44 ^b	44	Huffman et al. 1999
Cannabinol	211.2 ^{e,b}	126.4 ^e	Rhee et al. 1997
	308	96.3	Showalter et al. 1996
	1,130	301	Felder et al. 1995
CP56667	61.7	23.6	Showalter et al. 1996
<i>R</i> -(+)-WIN55212	9.94 ^b	16.2 ^b	Rinaldi-Carmona et al. 1994
	4.4 ^{a,b}	1.2 ^{a,b}	Hillard et al. 1999

Table 2. (continued)

Agonist	CB ₁ <i>K_i</i> value (nM)	CB ₂ <i>K_i</i> value (nM)	Reference
	1.89	0.28	Showalter et al. 1996
	62.3	3.3	Felder et al. 1995
	123	4.1	Shire et al. 1996
	9.87	0.29	Iwamura et al. 2001
CB₂-selective agonists in order of increasing CB₂/CB₁ selectivity			
AM1241	280 ^b	3.4 ^c	Ibrahim et al. 2003
3-(1' ¹ -dimethylbutyl)-1-deoxy-Δ ⁸ -THC (JWH-133)	677 ^b	3.4	Huffman et al. 1999
L-759633	1,043	6.4	Ross et al. 1999a
	15,850	20	Gareau et al. 1996
L-759656	529 ^b	35	Huffman et al. 1999
	713 ^b	57	Huffman et al. 2002
	4,888	11.8	Ross et al. 1999a
	>20,000	19.4	Gareau et al. 1996
HU-308	>10,000 ^{e,b}	22.7 ^{e,d}	Hanus et al. 1999

See Figs. 1 to 9 for the structures of the compounds listed in this table.

DMH, dimethylheptyl; ND, not determined; THC, tetrahydrocannabinol.

^aWith phenylmethylsulphonyl fluoride (PMSF) in order to inhibit enzymic hydrolysis.

^bBinding to rat cannabinoid receptors on transfected cells or on brain (CB₁) or spleen tissue (CB₂).

^cBinding to mouse brain (CB₁) or spleen tissue (CB₂).

^dSpecies unspecified. All other data from experiments with human cannabinoid receptors.

^eDisplacement of [³H]HU243 from CB₁- and CB₂-specific binding sites.

^fDisplacement of [³H]BAY-38-7271 from CB₁- and CB₂-specific binding sites.

tors to which at least some CB₁ and/or CB₂ receptor ligands can bind (Sect. 4). Radiolabelled probes for single photon emission computed tomography (SPECT) or positron emission tomography (PET) have also been developed (reviewed in Gifford et al. 2002; see also the chapter by Lindsey et al., this volume).

2.2

In Vitro Functional Bioassays

2.2.1

Assays Using Whole Cells or Cell Membranes

The most commonly employed assays using whole cells or cell membranes are the [³⁵S]guanosine-5'-O-(3-thiotriphosphate) ([³⁵S]GTPγS) binding assay and the cyclic AMP assay. The first measures cannabinoid receptor agonist-stimulated binding to G proteins of the hydrolysis-resistant GTP analogue, [³⁵S]GTPγS, whereas the cyclic AMP assay relies on cannabinoid receptor-mediated inhibition (usual effect) or enhancement of basal or drug-induced cyclic AMP production

Table 3. K_i values of cannabinoid receptor antagonists/inverse agonists for the in vitro displacement of [3 H]CP55940 from CB₁- and CB₂-specific binding sites

Ligand	CB ₁ K_i value (nM)	CB ₂ K_i value (nM)	Reference
CB₁-selective antagonists/inverse agonists			
NESS 0327	0.00035 ^a	21 ^a	Ruiu et al. 2003
SR141716A	11.8	13,200	Felder et al. 1998
	11.8	973	Felder et al. 1995
	12.3	702	Showalter et al. 1996
	5.6	>1,000	Rinaldi-Carmona et al. 1994
	1.98 ^b	>1,000 ^b	Rinaldi-Carmona et al. 1994
AM281	1.8 ^a	514 ^a	Ruiu et al. 2003
	12 ^b	4,200 ^a	Lan et al. 1999a
AM251 (compound 12)	7.49 ^b	2,290 ^a	Lan et al. 1999b
LY320135	141	14,900	Felder et al. 1998
CB₂-selective antagonists/inverse agonists			
AM 630	5,152	31.2	Ross et al. 1999a
SR144528	437	0.60	Rinaldi-Carmona et al. 1998
	305 ^b	0.30 ^b	Rinaldi-Carmona et al. 1998
	>10,000	5.6	Ross et al. 1999a
	70 ^a	0.28 ^a	Ruiu et al. 2003
	50.3	1.99	Iwamura et al. 2001

See Figs. 10 and 11 for the structures of the compounds listed in this table.

^aBinding to mouse brain (CB₁) or spleen tissue (CB₂).

^bBinding to rat cannabinoid receptors on transfected cells or on brain (CB₁) or spleen tissue (CB₂).

All other data from experiments with human cannabinoid receptor.

(reviewed in Howlett et al. 2002; Pertwee 1997, 1999a). Both assays can be performed with membranes obtained from brain tissue or from cultured cells that express CB₁ or CB₂ receptors either naturally or after transfection. In addition, the cyclic AMP assay can be performed with whole cells, including primary cultures of central neurons, and the [35 S]GTP γ S assay can be used in autoradiography experiments with tissue sections (Breivogel et al. 1997; Selley et al. 1996; Sim et al. 1995). The cyclic AMP assay is more sensitive than the [35 S]GTP γ S assay. Presumably this is because modulation of cyclic AMP production takes place further along the signalling cascade than [35 S]GTP γ S binding so that there is greater signal amplification. For the [35 S]GTP γ S assay, it is important to include guanosine diphosphate (GDP) and sodium chloride at appropriate concentrations (Breivogel et al. 1998; Selley et al. 1996; Sim et al. 1995). GDP increases the ratio of agonist-stimulated to basal [35 S]GTP γ S binding (signal-to-noise ratio) but also decreases the absolute levels of both agonist-stimulated and basal [35 S]GTP γ S binding. In addition, it magnifies the differences in efficacy exhibited in this assay by full and partial agonists (Savinainen et al. 2001). The signal-to-noise ratio in this bioassay can be further improved by including an adenosine A₁ receptor antagonist (Savinainen

et al. 2003). It has also proved possible to assay cannabinoid receptor agonists by exploiting their ability to increase intracellular free Ca^{2+} levels (CB_1 and CB_2 agonists) (Bisogno et al. 2000; Rubovitch et al. 2002; Sugiura et al. 1996, 1997, 2000; Suhara et al. 2001) or to inhibit lipopolysaccharide-induced release of tumour necrosis factor- α (CB_2 agonists) (Wroblewski et al. 2003). Some information about the pharmacological properties of cannabinoid receptor ligands has also been obtained using bioassays performed with cultured neurons that exploit the negative coupling of the CB_1 receptor to N- and P/Q-type calcium channels (reviewed in Pertwee 1997, 1999a).

2.2.2

Isolated Nerve–Smooth Muscle Preparations

Preparations in which cannabinoid receptor agonists can act through neuronal CB_1 receptors to produce a concentration-related inhibition both of electrically-evoked contractile transmitter release (Schlicker et al. 2003; Trendelenburg et al. 2000) and of the contractions caused by this release (reviewed in Howlett et al. 2002; Pertwee 1997; Pertwee et al. 1996a; Schlicker and Kathmann 2001) are called isolated nerve–smooth muscle preparations. The ones most commonly used are the mouse vas deferens and the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine. However, CB_1 receptor agonists also show activity in other isolated nerve-smooth muscle preparations, for example the rat vas deferens and the mouse urinary bladder. The usual measured response in these bioassays is inhibition of electrically evoked contractions, a response that can also be elicited in these tissues by agonists for several types of non-cannabinoid receptor. Consequently, to establish whether or not the production of such inhibition by a test compound is CB_1 receptor-mediated, it is necessary to measure the susceptibility of this compound to antagonism by a selective CB_1 antagonist. For the mouse vas deferens, an alternative strategy for meeting this objective has been to exploit the ability of a cannabinoid receptor agonist (Δ^9 -THC) to induce cannabinoid tolerance without affecting the sensitivity of the twitch response to inhibition by non-cannabinoids (Pertwee 1997).

2.3

In Vivo Bioassays

Probably the most commonly used in vivo bioassay is the mouse tetrad assay, in which the ability of a test compound to produce four effects in the same animal is determined. These effects, hypokinesia, hypothermia, catalepsy in the Pertwee ring test and antinociception in the tail-flick or hot plate test, are usually produced by a CB_1 receptor agonist over a relatively narrow dose range (reviewed in Howlett et al. 2002; Martin et al. 1995). One or other of these effects can be produced by some centrally active non- CB_1 receptor agonists or antagonists. However, when performed together, the tetrad tests provide at least some degree of

selectivity since, in contrast to established CB₁ receptor agonists, many other classes of centrally active agent lack activity in at least one of the tests (Wiley and Martin 2003). This feature of the tetrad assay was particularly important when it was first devised, as selective CB₁ receptor antagonists had still to be developed. Now that such antagonists are available (Sect. 3.2), there is less need for a bioassay with CB₁ receptor selectivity. Some non-CB₁ receptor ligands do show activity in all four tetrad tests. These include stearyl ethanolamide (Maccarrone et al. 2002), the anandamide analogue, O-2093 (Di Marzo et al. 2002), metabolites of anandamide (reviewed in Pertwee and Ross 2002) and certain anti-psychotic agents (Wiley and Martin 2003). Moreover, although the endocannabinoid anandamide shows cannabimimetic activity in the mouse tetrad assay, it is only antagonized by SR141716A when protected from enzymic hydrolysis (reviewed in Pertwee and Ross 2002). However, other CB₁ receptor agonists do show susceptibility to antagonism by SR141716A in this bioassay (reviewed in Howlett et al. 2002).

Other *in vivo* bioassays for CB₁ receptor agonists include the dog static ataxia test, the monkey behavioural test, the rat catalepsy test and the drug discrimination test, which is usually carried out with monkeys, rats or pigeons (reviewed in Howlett et al. 2002; Martin et al. 1995). The potencies shown by some cannabinoids in drug discrimination experiments performed with rats have been found to correlate well with their psychoactive potencies in humans (Balster and Prescott 1992). *In vivo* bioassays that provide measures of other CB₁ receptor-mediated effects in animals, for example changes in memory, have also been developed (reviewed in Howlett et al. 2002; see also the chapter by Riedel and Davies, this volume). However, these have not been used widely for characterizing novel cannabinoid receptor ligands. Methods for evaluating cannabinoids in humans have also been developed (Howlett et al. 2002).

2.4

Cannabinoid Receptor Knockout Mice

One important advance has been the development of transgenic CB₁^{-/-}, CB₂^{-/-} and CB₁^{-/-}/CB₂^{-/-} mice that lack CB₁, CB₂ or both CB₁ and CB₂ receptors (reviewed in Howlett et al. 2002; see also the chapters by Abood and by Valverde et al., this volume). The availability of such animals provides a useful additional method for establishing whether or not responses to test compounds are CB₁ and/or CB₂ receptor mediated and, indeed, an important means of detecting the presence of new types of cannabinoid receptor (Sect. 4.1). Cannabinoid receptor knockout mice are also being used to help determine the physiological roles of CB₁ and CB₂ receptors.

3 CB₁ and CB₂ Cannabinoid Receptor Ligands

3.1 Cannabinoid Receptor Agonists

In terms of chemical structure, established cannabinoid receptor agonists fall essentially into four main groups: classical, nonclassical, aminoalkylindole and eicosanoid (reviewed in Howlett et al. 2002; Pertwee 1999a).

- The classical group consists of dibenzopyran derivatives that are either cannabis-derived compounds (phytocannabinoids) or their synthetic analogues. Notable examples are the phytocannabinoids Δ^9 -THC, Δ^8 -THC and cannabiol (Fig. 1), and the synthetic cannabinoids, 11-hydroxy- Δ^8 -THC-dimethylheptyl (HU-210), JWH-133, L-759633, L-759656, L-nantradol and desacetyl-L-nantradol (Figs. 4 and 5).

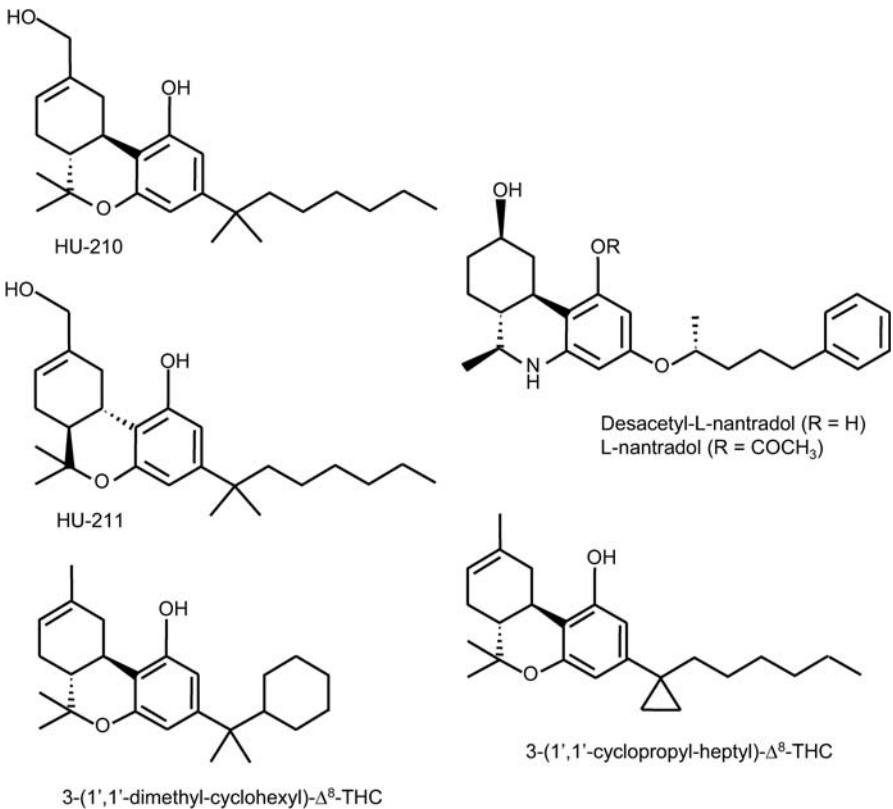


Fig. 4. The structures of five synthetic classical cannabinoids

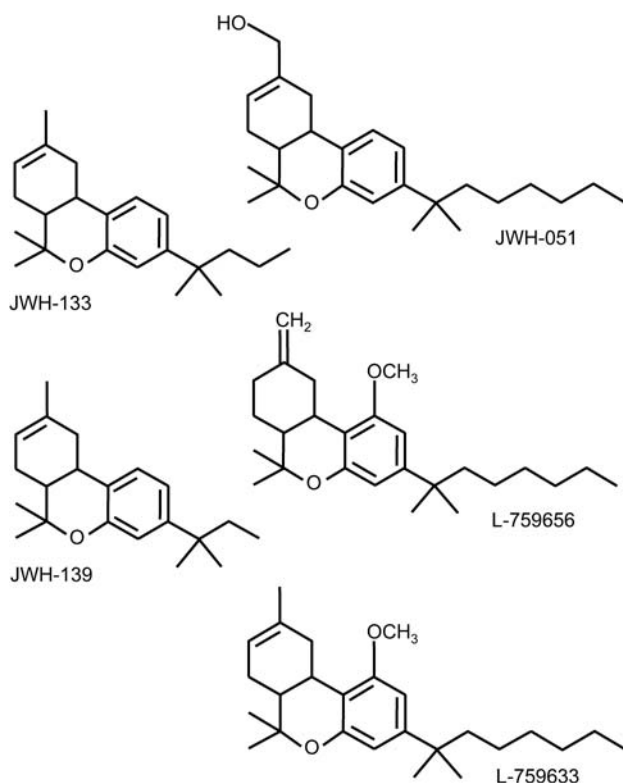


Fig. 5. The structures of four nonclassical cannabinoids

- Nonclassical cannabinoids consist of bicyclic and tricyclic analogues of Δ^9 -THC that lack a pyran ring; examples include CP55940, CP47497, CP55244 and HU-308 (Fig. 6). They are, therefore, closely related to the classical cannabinoids.
- In contrast, the aminoalkylindole group of cannabinoid receptor agonists (Fig. 7) have structures that are completely different from those of other cannabinoids. Indeed, results from experiments performed with wild-type and mutant CB₁ receptors (Chin et al. 1998; Petit et al. 1996; Song and Bonner 1996; Tao and Abood 1998) suggest that *R*-(+)-WIN55212 (WIN55212-2), the most widely investigated of the aminoalkylindoles, binds differently to the CB₁ receptor than classical, nonclassical or eicosanoid cannabinoids, albeit it in a manner that still allows mutual competition between *R*-(+)-WIN55212 and non-aminoalkylindole cannabinoids for binding sites on the wild-type receptor.
- Members of the eicosanoid group of cannabinoid receptor agonists have markedly different structures both from the aminoalkylindoles and from classical and nonclassical cannabinoids. Important members of this group are the endocannabinoids, arachidonylethanolamide (anandamide), *O*-arachidonylethanolamine (virodhamine), 2-arachidonoyl glycerol and 2-arachidonoyl glyceryl

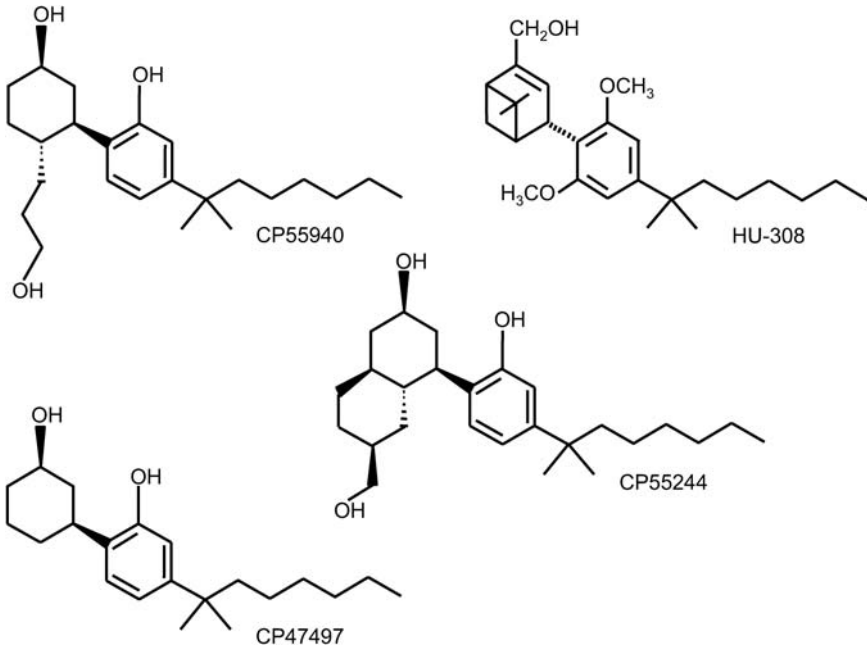


Fig. 6. The structures of four nonclassical cannabinoids. The (+)-enantiomer of CP55940 is CP56667

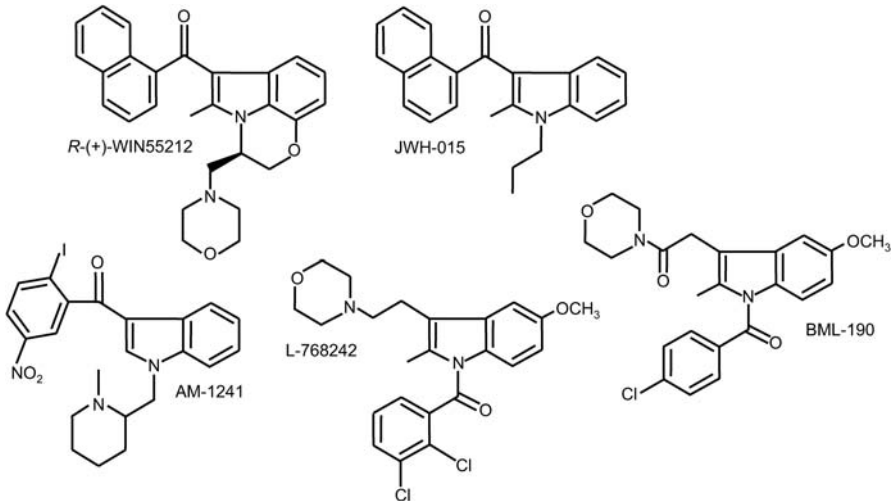


Fig. 7. The structures of *R*-(+)-WIN55212, JWH-015, AM1241, L-768242 and BML-190

ether (noladin ether) (Fig. 3) and several synthetic analogues of anandamide, including *R*-(+)-methanandamide, arachidonyl-2'-chloroethylamide (ACEA), arachidonylcyclopropylamide (ACPA), O-689 and O-1812 (Fig. 8) (Howlett et al. 2002; Pertwee 1999a; Porter et al. 2002).

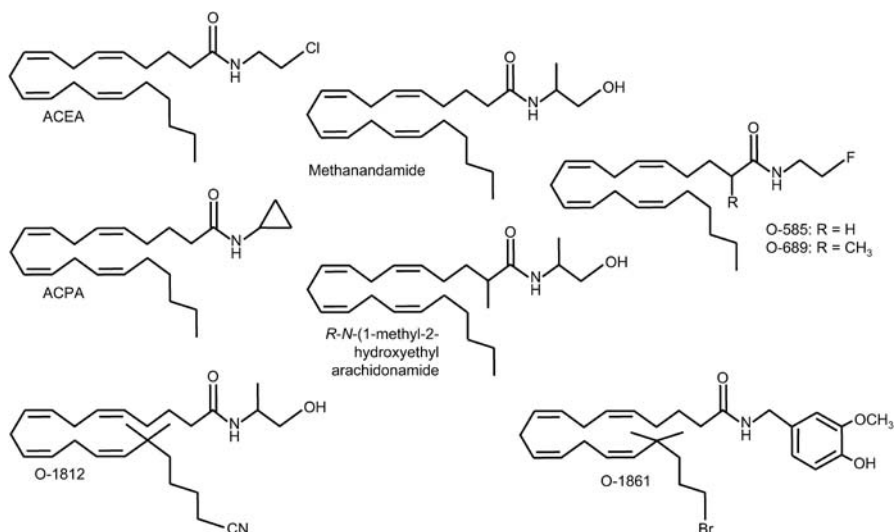


Fig. 8. The structures of eight structural analogues of anandamide

Many cannabinoid receptor agonists exhibit marked stereoselectivity in pharmacological assays, reflecting the presence of chiral centres in these compounds (reviewed in Howlett et al. 2002). Classical and nonclassical cannabinoids with the same absolute stereochemistry as $(-)\text{-}\Delta^9\text{-THC}$ at 6a and 10a, *trans* (6aR, 10aR), are more active than their *cis* (6aS, 10aS) enantiomers, whilst *R*-(+)-WIN55212 is more active than *S*-(-)-WIN55212. Although anandamide does not contain any chiral centres, some of its synthetic analogues do. One of these is methanandamide, the *R*-(+)-isomer of which exhibits nine times higher affinity for CB₁ receptors than the *S*-(-)-isomer (Abadji et al. 1994).

Several cannabinoid receptor agonists bind more or less equally well to CB₁ and CB₂ receptors (Table 2), although they do exhibit different relative intrinsic activities at these receptors. Among these are HU-210, CP55940, *R*-(+)-WIN55212, $(-)\text{-}\Delta^9\text{-THC}$, anandamide and 2-arachidonoyl glycerol (reviewed in Howlett et al. 2002; Pertwee 1999a).

- HU-210 has particularly high affinity for both CB₁ and CB₂ receptors. It also exhibits high relative intrinsic activities at these receptors. Indeed, it is remarkably potent as a cannabinoid receptor agonist and exhibits an exceptionally long duration of action in vivo. The marked affinity and efficacy that HU-210 shows at cannabinoid receptors is due largely to the replacement of the pentyl side chain of $\Delta^8\text{-THC}$ with a dimethylheptyl group.
- CP55940 and *R*-(+)-WIN55212 have CB₁ and CB₂ relative intrinsic activities of the same order as those of HU-210 and, although they have lower CB₁ and CB₂ affinities than HU-210, are still reasonably potent as they bind to these receptors at concentrations in the low nanomolar range.

- (-)- Δ^9 -THC has lower CB₁ and CB₂ affinities and relative intrinsic activities than HU-210, CP55940 or R-(+)-WIN55212. Whilst it behaves as a partial agonist at both these receptor types, it exhibits less efficacy at CB₂ than at CB₁ receptors to the extent that in one bioassay system it has been found to behave as a CB₂ receptor antagonist (Bayewitch et al. 1996). (-)- Δ^9 -THC can also produce CB₁ receptor antagonism. Thus, it has been found to oppose CB₁ receptor activation by the higher efficacy agonist, 2-arachidonoyl glycerol, in hippocampal cultures that may have contained neurons with rather low CB₁ receptor density (Kelley and Thayer 2004). This it did with an IC₅₀ of 42 nM, which is close to its reported CB₁ K_i values (Table 2).
- Anandamide resembles (-)- Δ^9 -THC in its affinity for CB₁ receptors, in behaving as a CB₁ and CB₂ receptor partial agonist (Gonsiorek et al. 2000; Hillard 2000; Mackie et al. 1993; Savinainen et al. 2001; Sugiura et al. 1996, 2000) and in having lower CB₂ than CB₁ intrinsic activity (reviewed in Howlett et al. 2002; Pertwee 1999a). It has also been found that, like (-)- Δ^9 -THC, anandamide can behave as a CB₂ receptor antagonist in at least one bioassay system (Gonsiorek et al. 2000). In contrast to R-(+)-WIN55212, which has slightly higher CB₂ than CB₁ affinity, anandamide binds marginally more readily to CB₁ than to CB₂ receptors.
- 2-Arachidonoyl glycerol is known to activate both CB₁ and CB₂ receptors. It binds about equally well to both receptor types (Table 2) and has been reported to exhibit greater CB₁ intrinsic activity but less CB₁ potency than CP55940 and greater CB₁ intrinsic activity and potency than anandamide (Gonsiorek et al. 2000; Savinainen et al. 2001, 2003; Sugiura et al. 1996). This endocannabinoid also has greater CB₂ potency than anandamide or 1-arachidonoyl glycerol (Gonsiorek et al. 2000; Sugiura et al. 2000).

One recently developed synthetic cannabinoid receptor agonist that interacts almost as well with CB₂ as with CB₁ receptors (Tables 1 and 2) is BAY 38-7271 (De Vry and Jentzsch 2002; Mauler et al. 2002, 2003). This compound has a structure that is not classical, non-classical, aminoalkylindole or eicosanoid (Fig. 9).

Phytocannabinoids other than Δ^9 -THC that are known to activate cannabinoid receptors are (-)- Δ^8 -THC and cannabinal (reviewed in Pertwee 1999a). Of these, (-)- Δ^8 -THC resembles (-)- Δ^9 -THC both in its CB₁ and CB₂ receptor affinities (Table 2) and in its relative intrinsic activity at the CB₁ receptor (Gérard et al. 1991; Howlett and Fleming 1984; Matsuda et al. 1990). Cannabinal also behaves as a partial agonist at CB₁ receptors but has even less relative intrinsic activity than (-)- Δ^9 -THC (Howlett 1987; Matsuda et al. 1990; Petit et al. 1997, 1998). Whilst there is one report that cannabinal activates CB₂ receptors in the cyclic AMP assay more effectively than Δ^9 -THC (Rhee et al. 1997), there is another that in the GTP γ S binding assay, it behaves as a CB₂ receptor inverse agonist (MacLennan et al. 1998).

As to the endocannabinoid virodhamine, Porter et al. (2002) have shown that this activates both CB₁ and CB₂ receptors. Their experiments with transfected cells yielded CB₁ and CB₂ EC₅₀ values in the GTP γ S binding assay of 1.9 and 1.4 μ M, respectively, for this endocannabinoid, indicating it to be less potent than anandamide, 2-arachidonoyl glycerol or R-(+)-WIN55212. The CB₂ intrinsic

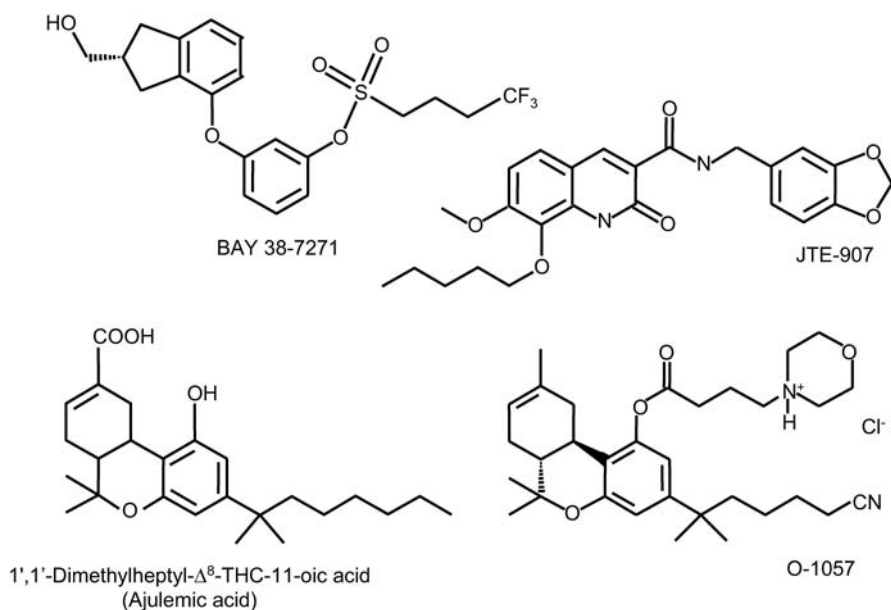


Fig. 9. The structures of BAY 38-7271, JTE-907, ajulemic acid and O-1057

activity of virodhamine matched that of anandamide which, however, behaved as a full agonist in this investigation, suggesting that the CB_2 expression level of the cell line used may have been rather high. In contrast, the CB_1 intrinsic activity of virodhamine was less than that of anandamide, and indeed it was found that virodhamine could attenuate anandamide-induced activation of CB_1 receptors. No binding data are yet available for virodhamine.

Turning now to potent cannabinoid receptor agonists that interact more readily with CB_1 or CB_2 receptors, a number of these have been developed. The starting point for all current CB_1 -selective agonists has been anandamide. Thus, results from binding experiments have shown that it is possible to enhance the marginal CB_1 selectivity exhibited by anandamide by replacing a hydrogen atom on the 1' or 2 carbon with a methyl group to form *R*-(+)-methanandamide or O-689 (Fig. 8) (Abadji et al. 1994; Showalter et al. 1996). As well as increasing CB_1 selectivity, insertion of a methyl group on the 1' or 2 carbon of anandamide increases resistance to the hydrolytic action of fatty acid amide hydrolase (FAAH) (Abadji et al. 1994; Adams et al. 1995). Anandamide analogues that exhibit particularly marked CB_1 -selectivity in binding assays are ACEA, ACPA and a cyano analogue of methanandamide (O-1812) (Table 2; Fig. 8). All three behave as potent CB_1 receptor agonists (Di Marzo et al. 2001; Hillard et al. 1999). O-1812 appears to lack significant susceptibility to hydrolysis by FAAH, presumably because it resembles *R*-(+)-methanandamide in having a methyl group attached to its 1'-carbon. ACEA and ACPA, which do not have the 1'-carbon methyl substituent of *R*-(+)-methanandamide, show no sign of reduced susceptibility to enzymic hy-

Table 4. K_i values of certain other ligands for the in vitro displacement of [3 H]CP55940 or [3 H]HU243^a from CB₁- and CB₂-specific binding sites

Ligand	CB ₁ K_i value (nM)	CB ₂ K_i value (nM)	Reference
CB₁-selective ligands in order of decreasing CB₁/CB₂ selectivity			
<i>R-N</i> -(1-methyl-2-hydroxyethyl)- 2- <i>R</i> -methyl-arachidonamide	7.42 ^{b,c}	1,952 ^d	Goutopoulos et al. 2001
O-585	8.6 ^b	324 ^b	Showalter et al. 1996
O-689	5.7 ^b	132 ^b	Showalter et al. 1996
Ligands without any marked CB ₁ or CB ₂ selectivity			
Ajulemic acid (CT-3)	32.3 ^{a,c}	170.5 ^a	Rhee et al. 1997
11-OH-cannabinol-DMH	0.1 ^{a,c}	0.2 ^a	Rhee et al. 1997
3-(1',1'-dimethyl-cyclohexyl)- Δ^8 -THC	0.57	0.65	Krishnamurthy et al. 2003
11-OH-cannabinol	38 ^{a,c}	26.6 ^a	Rhee et al. 1997
Δ^9 -THC-DMH	0.241 ^{a,c}	0.199 ^a	Rhee et al. 1997
Cannabinol-DMH	2 ^{a,c}	1.5 ^a	Rhee et al. 1997
Cannabidiol	4,350	2,860	Showalter et al. 1996
	>10 ^{a,c}	>10 ^{a,e}	Bisogno et al. 2001
11-OH- Δ^8 -THC	25.8 ^{a,c}	7.4 ^a	Rhee et al. 1997
1-Deoxy- Δ^8 -THC-DMH	23 ^c	2.9	Huffman et al. 1996
3-(1',1'-cyclopropyl-heptyl)- Δ^8 -THC	0.44 ^c	0.86 ^d	Papahatjis et al. 2002
O-1184	5.25	7.41	Ross et al. 1999b
<i>cis</i> (6a <i>S</i> , 10a <i>S</i>)-3-(1',1'-DMH)- 11-hydroxy- Δ^8 -THC (HU-211)	1,990	>10,000	Showalter et al. 1996
Abnormal-cannabidiol	>10,000	>10,000	Showalter et al. 1996
CB₂-selective ligands in order of increasing CB₁/CB₂ selectivity			
JWH-015	383	13.8	Showalter et al. 1996
1-Deoxy-11-hydroxy- Δ^8 -THC-DMH (JWH-051)	1.2 ^c	0.032	Huffman et al. 1996
JTE-907	2,370	35.9	Iwamura et al. 2001
L-768242	1,917	12	Gallant et al. 1996
3-(1'1'-dimethylpropyl)- 1-deoxy- Δ^8 -THC (JWH-139)	2,290 ^c	14	Huffman et al. 1999
3-(1'1'-dimethylhexyl)- 1-methoxy- Δ^8 -THC	3,134 ^c	18	Huffman et al. 2002
1-Deoxy- Δ^8 -THC	>10,000 ^c	32	Huffman et al. 1999

See Figs. 1, 4, 5, 7, 8, 9, 11 and 12 for the structures of some of the compounds listed in this table. DMH, dimethylheptyl; ND, not determined; THC, tetrahydrocannabinol.

^bWith phenylmethylsulfonyl fluoride (PMSF) in order to inhibit enzymic hydrolysis.

^cBinding to rat cannabinoid receptors on transfected cells or on brain (CB₁) or spleen tissue (CB₂).

^dBinding to mouse brain (CB₁) or spleen tissue (CB₂).

^eSpecies unspecified. All other data from experiments with human cannabinoid receptors.

drolisis. Although insertion of this group into ACEA does markedly reduce the susceptibility of this molecule to FAAH-mediated hydrolysis, it also decreases the affinity of ACEA for CB₁ receptors by about 14-fold (Jarranian et al. 2000). *R-N*-(1-

methyl-2-hydroxyethyl)-2-*R*-methyl-arachidonamide, which also exhibits marked CB₁-selectivity in binding assays (Table 4), has less metabolic stability than *R*-(+)-methanandamide (Goutopoulos et al. 2001). Another CB₁-selective agonist of note is the endocannabinoid 2-arachidonyl glyceryl ether (Hanus et al. 2001), the CB₁ intrinsic activity of which has been reported to match that of CP55940 and to be less than that of 2-arachidonoyl glycerol. 2-Arachidonoyl glyceryl ether exhibits less potency at CB₁ receptors than either CP55940 or 2-arachidonoyl glycerol (Savinainen et al. 2001, 2003; Suhara et al. 2000, 2001).

The best CB₂-selective agonists to have been developed to date are all non-icosanoid cannabinoids (Howlett et al. 2002; Ibrahim et al. 2003; Pertwee 1999a). They include the classical cannabinoids, L-759633, L-759656 and JWH-133, the non-classical cannabinoid HU-308, and the aminoalkylindole AM1241 (Figs. 5, 6 and 7). All these ligands bind more readily to CB₂ than to CB₁ receptors (Table 2) and have also been shown to behave as potent CB₂-selective agonists in functional bioassays (Hanus et al. 1999; Ibrahim et al. 2003; Pertwee 2000; Ross et al. 1999a).

One other cannabinoid receptor agonist of note is 3-(5'-cyano-1',1'-dimethylpentyl)-1-(4-*N*-morpholinobutyryloxy)- Δ^8 -THC hydrochloride (O-1057). Thus, unlike all established cannabinoid receptor agonists, this is readily soluble in water and yet, compared to CP55940, its potency in the cyclic AMP assay is just 2.9 times less at CB₁ receptors and 6.5 times less at CB₂ receptors (Pertwee et al. 2000). The finding that it is possible to solubilize a cannabinoid and yet retain pharmacological activity has important implications for cannabinoid delivery not only in the laboratory but also in the clinic. As to structure-activity relationships for cannabinoid receptor agonists, the salient features of these have been well described elsewhere (Howlett et al. 2002; Pertwee 1999a). Recent findings of special interest are that the CB₁ and CB₂ affinities of Δ^8 -THC can be greatly enhanced both by replacing its C3 pentyl side chain with a 1',1'-dimethyl-1'-cyclohexyl moiety (Fig. 4; Table 4) (Krishnamurthy et al. 2003) and by changing this side chain from pentyl to heptyl and introducing a cyclopropyl group at the 1' position (Fig. 4; Table 4) (Papahatjis et al. 2002).

3.2

Cannabinoid CB₁ and CB₂ Receptor Antagonists

3.2.1

Selective CB₁ Receptor Antagonists

The first selective CB₁ receptor antagonist, the diarylpyrazole SR141716A (Fig. 10), was developed by Sanofi Recherche (Rinaldi-Carmona et al. 1994). This readily prevents or reverses effects induced by cannabinoids at CB₁ receptors, both in vitro and in vivo (reviewed in Howlett et al. 2002; Pertwee 1997). It binds with significantly higher affinity to CB₁ than CB₂ receptors (Table 3), lacks significant affinity for a wide range of non-cannabinoid receptors and does not exhibit detectable agonist activity at CB₁ and CB₂ receptors (Hirst et al. 1996; Rinaldi-Carmona et al. 1994, 1996a,b; Shire et al. 1996). Other established CB₁-selective antagonists are

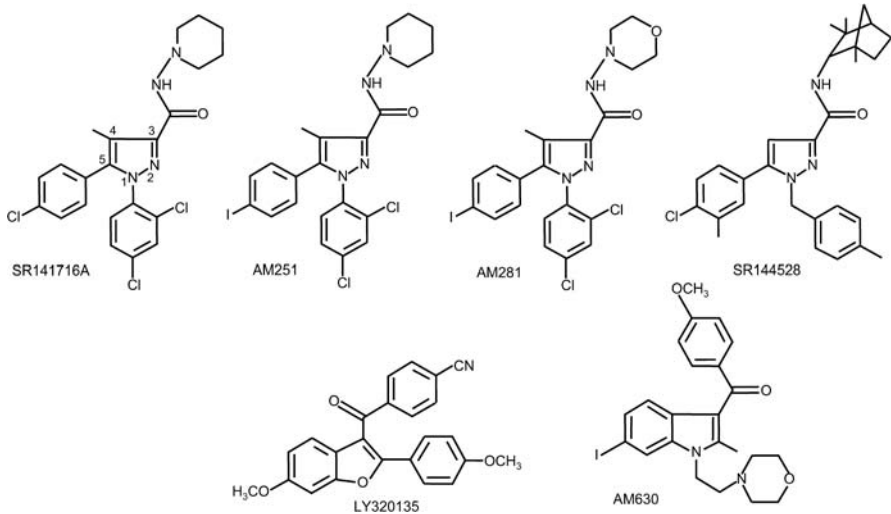


Fig. 10. The structures of several CB₁- or CB₂-selective antagonists/inverse agonists

LY320135, AM251 and AM281 (Fig. 10). LY320135, developed by Eli Lilly, also binds with lower affinity to CB₁ than CB₂ receptors (Table 3). However, its CB₁ affinity is less than that of SR141716A. Moreover, at concentrations in the low micromolar range, LY320135 also binds to muscarinic and 5-hydroxytryptamine (5-HT)₂ receptors ($K_i < 10 \mu\text{M}$) and, at higher concentrations, to histamine H₁ receptors ($K_i = 12.9 \mu\text{M}$), α_1 - and α_2 -adrenoceptors and dopamine D₁ and D₂ receptors (Felder et al. 1998). AM251 and AM281 are both structural analogues of SR141716A. They have been found to displace [³H]SR141716A from binding sites on mouse cerebellar membranes with respectively three and eight times less potency than SR141716A (Gatley et al. 1998), and both compounds have also been shown to bind more readily to CB₁ than CB₂ receptors (Table 3). There are numerous reports that, like SR141716A, AM251 and AM281 can attenuate *in vivo* or *in vitro* responses to established cannabinoid receptor agonists (e.g. Cosenza et al. 2000; Gifford et al. 1997; Hájos and Freund 2002a; Lan et al. 1999a; Simoneau et al. 2001).

Although SR141716A is CB₁-selective, it is not CB₁-specific. Thus, results from binding experiments indicate that whilst it may be reasonable to assume that concentrations of this ligand in the low or mid nanomolar range will interact mainly with the CB₁ receptors when it is applied to tissues that contain both CB₁ and CB₂ receptors, this is not so for higher concentrations of SR141716A (Table 3). Results obtained *in vitro* from functional bioassays also suggest that CB₁ receptors are not the only pharmacological targets with which this compound can interact at micromolar concentrations. For example, it has been found that SR141716A can stimulate extracellular-signal-regulated protein kinase (ERK) at 1 μM (Berdyshev et al. 2001) and antagonize anandamide-induced vasodilation in the mesenteric arteries of CB₁^{-/-} mice at 1 and 5 μM (Járai et al. 1999). In addition there are reports that at concentrations above 1 μM , SR141716A can both block and activate

transient receptor potential vanilloid type 1 (TRPV1) receptors (previously known as VR1 receptors), suggesting that it may be a TRPV1 receptor partial agonist (De Petrocellis et al. 2001a; Zygmunt et al. 1999), block adenosine A₁ receptors (as can AM251) (Savinainen et al. 2003), oppose vasorelaxation induced by acetylcholine in ring preparations of rabbit precontracted isolated superior mesenteric arteries (Chaytor et al. 1999) and by bradykinin in human precontracted myometrial small arteries (Kenny et al. 2002), and block potassium and L-type calcium channels in rat isolated mesenteric arteries (White and Hiley 1998) and gap junctions between COS-7 cells (Chaytor et al. 1999).

Unexpectedly, in spite of the close similarity between the structures of AM251, AM281 and SR141716A, differences in their pharmacological profiles have been detected in vitro in experiments with cardiovascular tissue (reviewed in Pertwee 2004a). It has also been found that the ability of *R*-(+)-WIN55212 to reduce glutamatergic transmission is opposed by 1 μM SR141716A in CB₁^{-/-} mouse hippocampal slices but not by 2 μM AM251 in rat hippocampal slices (Hájos and Freund 2002a; Hájos et al. 2001).

3.2.2

Selective CB₂ Receptor Antagonists

The most important selective CB₂ receptor antagonists are the diarylpyrazole SR144528 and the aminoalkylindole 6-iodopravadoline (AM630) (Fig. 10). Both bind with markedly higher affinity to CB₂ than CB₁ receptors (Table 3) and prevent or reverse in vitro effects mediated by CB₂ receptors (Portier et al. 1999; Rinaldi-Carmona et al. 1998; Ross et al. 1999a). Evidence also exists that on the one hand, SR144528 lacks significant affinity for a wide range of established non-cannabinoid receptors (Rinaldi-Carmona et al. 1998), and on the other hand it is an antagonist for a putative CB₂-like receptor that is activated by palmitoylethanolamide, a ligand that does not have significant CB₂ receptor affinity (Sect. 4.1.3). Interestingly, it has proved possible to develop diarylpyrazoles with even greater CB₂ selectivity and affinity than SR144528 (Mussinu et al. 2003). This has been achieved by making these molecules less flexible.

Turning now to AM630, particularly with regard to its behaviour at the CB₁ receptor, there are several reports that when administered at concentrations in the micromolar range, it exhibits the mixed agonist-antagonist properties typical of a weak partial agonist for this receptor (reviewed in Pertwee 1999a). However, there are also reports that AM630 can behave as a CB₁ receptor inverse agonist (Landsman et al. 1998; Vásquez et al. 2003).

3.3

Inverse Agonism at Cannabinoid Receptors

There is good evidence that when administered by itself in vivo or in vitro, SR141716A is capable of producing inverse cannabimimetic effects, i.e. effects

that are opposite in direction to those produced by the activation of CB₁ receptors (reviewed in Pertwee 2003). There are also reports that such inverse effects can be induced by the other cannabinoid receptor antagonists described in Sect. 3.2: AM251 (Vásquez et al. 2003), AM281 (Cosenza et al. 2000; Gifford et al. 1997; Izzo et al. 2000; Vásquez et al. 2003), LY320135 (Felder et al. 1998) and AM630 (Sect. 3.2.2) at CB₁ receptors and SR144528 (Portier et al. 1999; Rinaldi-Carmona et al. 1998; Ross et al. 1999b), AM630 (New and Wong 2003; Ross et al. 1999a) and AM251 (New and Wong 2003) at CB₂ receptors. These effects include SR141716A- and AM281-induced hyperkinesia in rats and/or mice (Compton et al. 1996; Cosenza et al. 2000; Costa and Colleoni 1999) and the attenuation *in vitro* of CB₁ or CB₂ receptor signalling. Two other compounds, the CB₂-selective ligands JTE-907 and BML-190 (Figs. 7 and 9), also behave as CB₂ receptor inverse agonists (Iwamura et al. 2001; New and Wong 2003). However, whether JTE-907 or BML-190 produces antagonism at CB₂ receptors has not been reported.

Whereas some inverse cannabimimetic effects of SR141716A may be produced as a result of antagonism of responses to endogenously released endocannabinoids, there is evidence that others are not, prompting the hypothesis that this compound is an inverse agonist that can elicit responses at CB₁ receptors that are opposite in direction from those elicited by conventional agonists. This turn has been taken to indicate that CB₁ receptors can exist in two or more interchangeable conformations (reviewed in Pertwee 2003, 2005). More specifically, it has been proposed that these are (1) a constitutively active “on” state in which the receptors are functionally coupled to their effector mechanisms even in the absence of exogenously added or endogenously produced cannabinoid receptor agonists and (2) one or more “off” states in which the receptors are uncoupled from their effector mechanisms. According to this hypothesis, agonists increase the proportion of receptors in the “on” state, inverse agonists increase the proportion of receptors in the “off” state(s) and neutral antagonists leave the number of receptors in each state unchanged.

There is evidence that SR141716A exhibits greater potency in opposing effects induced by CB₁ agonists than in producing inverse effects at CB₁ receptors by itself (e.g. Sim-Selley et al. 2001). This raises the possibilities, first, that SR141716A may be a neutral CB₁ receptor antagonist at low concentrations that exhibits additional CB₁ inverse agonist activity only at higher concentrations, and secondly, that SR141716A may have two sites of action on the CB₁ receptor, one at which it displaces agonists to produce antagonism and another at which it somehow induces inverse agonism, perhaps through an allosteric mechanism (Sim-Selley et al. 2001).

Although it is likely that at least some of the inverse effects produced by SR144528 or AM630 at CB₂ receptors are also due to inverse agonism, no attempts have been made to establish this conclusively. It is noteworthy, therefore, that the finding that a maximal concentration of SR144528 enhances forskolin-stimulated cyclic AMP production by human (h)CB₂-transfected CHO cells considerably more than a maximal concentration of AM630 (Ross et al. 1999a,b) can be better explained in terms of inverse agonism at the CB₂ receptor than in terms of antagonism of endogenously released endocannabinoids. This is because the simplest explanation for this difference between the maximal inverse effects of these two ligands is that

SR144528 has greater inverse intrinsic activity than AM630. If this interpretation of the data is valid, it is of course an indication that just as the intrinsic activities of CB₁ and CB₂ receptor agonists can vary from compound to compound, so too the (inverse) intrinsic activities of cannabinoid receptor inverse agonists will not be the same for all such ligands.

Whilst there is little doubt that the presence of CB₁ receptors is a prerequisite for the production by SR141716A of many of its inverse cannabimimetic effects, it is noteworthy that this compound has been found to produce an effect on GTP γ S binding to whole brain membranes obtained from CB₁^{-/-} mice (enhancement) opposite to that produced by *R*-(+)-WIN55212 or anandamide (inhibition) (Breivogel et al. 2001). This finding supports the hypothesis that at least some apparent inverse effects of SR141716A may be induced at sites that are not located on CB₁ receptors (Sim-Selley et al. 2001). Indeed, it is already known that SR141716A not only binds to CB₂ receptors at concentrations in the high nanomolar range and above (Table 3) but also behaves as a CB₂ receptor inverse agonist at such concentrations, as measured by inhibition of [³⁵S]GTP γ S binding to hCB₂ receptors on CHO cell membranes (MacLennan et al. 1998).

3.4

Neutral Antagonism at Cannabinoid Receptors

An important recent pharmacological objective has been the development of cannabinoid receptor ligands for CB₁ and CB₂ receptors that completely lack both inverse agonist and agonist properties (neutral antagonists). One cannabinoid receptor ligand that comes close to being a neutral antagonist is 6'-azidohex-2'-yne- Δ^8 -THC (O-1184; Fig. 11 and Table 4), as this behaves as a high-affinity, low-efficacy agonist at CB₁ receptors and as a high-affinity, low-efficacy inverse agonist at CB₂ receptors, and as it produces potent antagonism of *R*-(+)-WIN55212 and CP55940 in the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine (Ross et al. 1998, 1999b). More recently, an analogue of SR141716A, NESS 0327, has been developed that behaves as a neutral CB₁ receptor antagonist and is markedly more potent and CB₁-selective than SR141716A (Table 3) (Ruii et al. 2003). This was achieved by reducing the molecule's flexibility through the introduction of a seven-membered ring (Fig. 11). Evidence has also emerged that insertion of a 6''-azidohept-2''-yne side chain into cannabidiol (Fig. 1) converts this molecule into a neutral cannabinoid receptor antagonist (Thomas et al. 2004). This compound, O-2654 (Fig. 11), has markedly higher affinity than cannabidiol for CB₁ receptors and antagonizes *R*-(+)-WIN55212-induced inhibition of electrically evoked contractions of the mouse isolated vas deferens in a competitive, surmountable manner with a K_B (85.7 nM) that is close to its K_i for displacing [³H]CP55940 from CB₁ receptors (114 nM). The conclusion that O-2654 may be a neutral antagonist is based on the observation that at concentrations of up to 10 μ M, it exhibits no detectable CB₁ agonist or inverse agonist properties in the mouse isolated vas deferens. Thus, unlike SR141716A (Pertwee et al. 1996b), O-2654 does not increase the amplitude of electrically evoked contractions of this

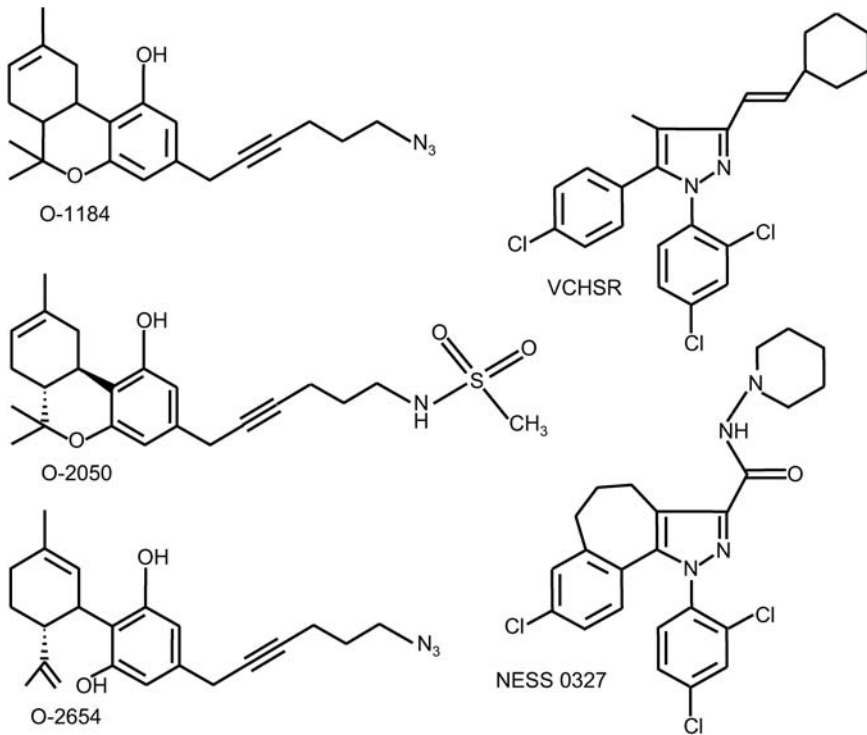


Fig. 11. The structure of O-1184 and of some putative neutral cannabinoid receptor antagonists

preparation. Nor does it share the ability of the CB_1 partial agonist, O-1184, to inhibit these contractions (Ross et al. 1999b). O-2050, a sulphonamide analogue of Δ^8 -THC with an acetylenic side chain also behaves as a neutral CB_1 receptor antagonist in the mouse vas deferens (Martin et al. 2002). Another compound that seems to be a neutral CB_1 antagonist is VCHSR (Fig. 11). This is an analogue of SR141716A that lacks hydrogen bonding capability in its C3 substituent region and has a CB_1 K_i value in the low nanomolar range. VCHSR (1 μ M) has been found to share the ability of SR141716A to attenuate *R*-(+)-WIN55212-induced inhibition of Ca^{2+} current in rat superior cervical ganglion neurons expressing the human CB_1 receptor but to differ from SR141716A in not affecting Ca^{2+} current in these neurons when administered by itself at 1 or 10 μ M (Hurst et al. 2002; Pan et al. 1998). In terms of the two-state model of inverse agonism (see Pertwee 2003, 2005 and Sect. 3.3), this finding suggests that preferential binding by SR141716A to the “off” state of the CB_1 receptor is determined by hydrogen bond formation between the C3 substituent of this molecule and the receptor. Further experiments are required to establish whether putative neutral antagonists, such as NESS 0327, O-2654 and O-2050, resemble SR141716A (Sect. 3.3) in exhibiting inverse agonist properties at concentrations above those at which they behave as neutral antagonists.

4 Other Pharmacological Targets for Cannabinoids in Mammalian Tissues

As discussed in greater detail elsewhere (Hájos and Freund 2002b; Howlett et al. 2002; Pertwee 1999b, 2004a; Pertwee and Ross 2002; Wiley and Martin 2002), evidence is emerging that in addition to CB₁ and CB₂ receptors, there are other pharmacological targets in mammalian tissues with which at least some established CB₁ and/or CB₂ receptor agonists can interact to elicit pharmacological responses.

4.1 Receptors

4.1.1 Vanilloid Receptors

It is now generally accepted that the endogenous CB₁/CB₂ receptor agonist, anandamide, and certain of its analogues are agonists for the TRPV1 receptor (reviewed in Howlett et al. 2002; Pertwee 2004a; Pertwee and Ross 2002; Ross 2003). This receptor is a non-selective cation channel that is present on sensory neurons in tissues such as skin, heart, blood vessels and lung, and an important consequence of its activation is the release of sensory neuropeptides that then produce effects such as pain, tachycardia, vasodilation and bronchoconstriction. It is noteworthy, however, that anandamide has less TRPV1 intrinsic activity than the well-known TRPV1 receptor agonist capsaicin (Ross 2003; Ross et al. 2001). *R*-(+)-methanandamide is even less potent or effective than anandamide at activating TRPV1 receptors (Ross et al. 2001; Zygmunt et al. 1999), whereas lipoxygenase metabolites of anandamide show greater potency at these receptors than their parent compound, at least in guinea-pig bronchus (Craib et al. 2001; Pertwee and Ross 2002). The TRPV1 receptor is not activated by 2-arachidonoyl glycerol or by non-eicosanoid CB₁/CB₂ receptor agonists (Zygmunt et al. 1999), although it is activated by micromolar concentrations of the phytocannabinoid cannabidiol (Bisogno et al. 2001). One compound that behaves as a potent agonist at both TRPV1 and CB₁ receptors is the synthetic anandamide analogue O-1861 (Fig. 8) (Di Marzo et al. 2001). TRPV1 and CB₁ receptors have opposite effects on calcium channel conductance, and there are several reports that in cells such as cultured dorsal root ganglion neurons that co-express these receptors, responses elicited by TRPV1 receptor activation can be opposed by the simultaneous activation of CB₁ receptors (Ahluwalia et al. 2003; Ellington et al. 2002; Millns et al. 2001; Richardson et al. 1998; Ross 2003). Unexpectedly, however, there is also a report that in human embryonic kidney cells co-transfected with CB₁ and TRPV1 receptors, activation of the CB₁ receptors increases the sensitivity of the TRPV1 receptors to subsequent (but not simultaneous) activation (Hermann et al. 2003). Under physiological conditions, TRPV1 receptors on primary sensory neurons are less sensitive to anandamide than CB₁ receptors (Németh et al. 2003; Tognetto et al. 2001). There is also evidence that anandamide production increases during inflammation, raising the possibility that

in healthy tissue, one role of anandamide may be to act through CB₁ receptors to oppose any increase in the excitability of sensory neurons, whilst in pathological states such as inflammation, anandamide concentrations and TRPV1 receptor sensitivity increase to the extent that anandamide-induced activation of TRPV1 receptors becomes sufficient to cause an increase in the excitability of sensory neurons (Ahluwalia et al. 2003). Although there is little doubt that anandamide is an endogenous agonist for CB₁ and CB₂ receptors, the question of whether it also serves as an endogenous TRPV1 agonist under normal or pathological conditions has still to be resolved. Also currently uncertain is the extent to which CB₁ and TRPV1 receptors are co-expressed on the same neurons (reviewed in Ross 2003).

4.1.2

CB₁ Receptor Subtypes

Shire et al. (1995) have isolated a spliced variant of CB₁ cDNA (CB_{1A}) from a human lung cDNA library. CB_{1A} mRNA is present in human brain tissue, its distribution pattern matching that of CB₁ mRNA. It has also been detected in peripheral tissues. The spliced variant resembles the CB₁ receptor in its affinity for Δ^9 -THC, CP55940 and *R*-(+)-WIN55212, and it also has at least two signal transduction mechanisms in common with the CB₁ receptor (Rinaldi-Carmona et al. 1996a). However, the central and peripheral concentrations of CB_{1A} mRNA are far below those of CB₁ mRNA (Shire et al. 1995). Onaivi et al. (1996) have discovered three distinct CB₁ mRNAs in brain tissue from C57BL/6 mice, although only one CB₁ receptor cDNA. C57BL/6 mice were less sensitive to the hypothermic and antinociceptive effects of Δ^9 -THC than two other mouse strains in which only one CB₁ mRNA was detectable.

Results from pharmacological experiments with rats and mice performed by Sandra Welch's group also suggest that there may be more than one subtype of CB₁ receptor (reviewed in Howlett et al. 2002; Pertwee 2001). In mouse experiments, for example, it was found that intraperitoneal SR141716A was more effective in opposing the antinociceptive effects of some CB₁ receptor agonists than of other such agonists when these were administered intrathecally and that intrathecal morphine interacted synergistically with intrathecal THC but not with intrathecal CP55940. Apparent differences between mouse cannabinoid receptors in brain and spinal cord were also detected.

4.1.3

CB₂-Like Receptors

It is possible that palmitoylethanolamide may produce antinociception in rat and mouse models of inflammatory or neuropathic pain by acting on a CB₂-like receptor (Calignano et al. 1998, 2001; Conti et al. 2002; Farquhar-Smith et al. 2002; Farquhar-Smith and Rice 2001; Helyes et al. 2003). The existence of such a receptor is supported by the finding that even though palmitoylethanolamide lacks significant CB₂ receptor affinity or efficacy (Griffin et al. 2000; Lambert et al. 1999;

Sheskin et al. 1997; Showalter et al. 1996), the antinociceptive effects of this fatty acid amide are opposed by SR144528. Evidence for CB₂-like receptors has also been obtained from experiments with the mouse vas deferens (Griffin et al. 1997). Other possibilities, i.e. that palmitoylethanolamide acts through CB₁ or TRPV1 receptors, can be ruled out. Thus, it produces antinociceptive effects that are not opposed by SR141716A (Calignano et al. 1998, 2001; Farquhar-Smith et al. 2002; Farquhar-Smith and Rice 2001) and it has been found not to attenuate nociceptive behaviour induced in mice by intraplantar injection of capsaicin (Calignano et al. 2001). Also, palmitoylethanolamide does not bind to or activate CB₁ receptors at concentrations below 1 or 10 μM (Devane et al. 1992; Felder et al. 1993; Griffin et al. 2000; Lambert et al. 1999; Showalter et al. 1996). Anandamide shares the ability of palmitoylethanolamide to induce antinociception in mice and rats. However, unlike palmitoylethanolamide, it has been found to be susceptible to SR141716A-induced antagonism and resistant to SR144528-induced antagonism in several pain models (Calignano et al. 1998, 2001; Farquhar-Smith and Rice 2001). Also, in contrast to palmitoylethanolamide, anandamide attenuates nociceptive behaviour induced in mice by intraplantar injection of capsaicin (Calignano et al. 2001). Another observation—that palmitoylethanolamide and anandamide interact synergistically rather than additively in the mouse formalin paw and abdominal stretch tests—also supports the hypothesis that they have different antinociceptive mechanisms (Calignano et al. 1998, 2001).

4.1.4

Neuronal Non-CB₁, Non-CB₂, Non-TRPV1 Receptors

Central G Protein-Coupled Receptors for Anandamide and R-(+)-WIN55212

Evidence for the presence of a G protein-coupled non-CB₁, non-CB₂ receptor for anandamide and R-(+)-WIN55212 has come from experiments in which it was found that [³⁵S]GTPγS binding to whole-brain membranes from CB₁^{-/-} C57BL/6 mice or to cerebellar homogenates from CB₁^{-/-} CD1 mice could be enhanced by these two cannabinoids (Breivogel et al. 2001; Di Marzo et al. 2000; Monory et al. 2002). Near maximal concentrations of anandamide and R-(+)-WIN55212 were not fully additive in their effects on [³⁵S]GTPγS binding to CB₁^{-/-} C57BL/6 brain membranes, supporting the hypothesis that these two agents were acting through a common mechanism (Breivogel et al. 2001). This putative receptor for anandamide and R-(+)-WIN55212 appears not to be a TRPV1 receptor (Sect. 4.1.1) or to resemble the proposed abnormal-cannabidiol receptor (Sect. 4.1.5) as neither of these pharmacological targets is R-(+)-WIN55212-sensitive and as the TRPV1 receptor is not G protein coupled. However, the possibility does remain that it may be a novel metabotropic “vanilloid-like” receptor (see below). The proposed new receptor also differs from established cannabinoid receptors in several ways.

- It is not sensitive to activation by the established CB₁/CB₂ receptor agonists, Δ^9 -THC, CP55940 or HU-210 (Breivogel et al. 2001; Di Marzo et al. 2000; Monory et al. 2002).
- It is not coupled to adenylate cyclase, at least in the cerebellum of CB₁^{-/-} CD1 mice (Monory et al. 2002).
- It differs from the CB₁ receptor in its central distribution pattern (Breivogel et al. 2001; Monory et al. 2002).
- SR141716A and SR144528 do not appear to be competitive antagonists for this putative receptor (Breivogel et al. 2001; Monory et al. 2002).
- There are no specific binding sites for [³H]CP55940 on CB₁^{-/-} C57BL/6 mouse brain membranes (Breivogel et al. 2001).

It has also been found that [³H]R-(+)-WIN55212 undergoes selective binding to CB₁^{-/-} C57BL/6 membranes obtained from brain areas in which R-(+)-WIN55212 enhances [³⁵S]GTP γ S binding (cerebral cortex, hippocampus and brain stem) (Breivogel et al. 2001). Furthermore, CB₁^{-/-} C57BL/6 brain areas that are unresponsive to R-(+)-WIN55212-induced enhancement of [³⁵S]GTP γ S binding seem to lack [³H]R-(+)-WIN55212 binding sites (Breivogel et al. 2001). It is noteworthy, however, that some WIN55212-sensitive brain areas of CB₁^{-/-} C57BL/6 mice (midbrain and diencephalon) and of CB₁^{-/-} CD1 mice (cerebellum) also seem to lack [³H]R-(+)-WIN55212 binding sites (Breivogel et al. 2001; Ledent et al. 1999; Monory et al. 2002). Although CB₁^{-/-} C57BL/6 mouse brain does contain specific binding sites for both [³H]SR141716A and [³H]R-(+)-WIN55212, these two binding site populations have different distribution patterns (Breivogel et al. 2001). This is further evidence that SR141716A lacks affinity for the proposed R-(+)-WIN55212/anandamide receptor.

A pharmacological property that the proposed R-(+)-WIN55212/anandamide receptor may share with the CB₁ receptor is the ability to mediate antinociception, catalepsy and hypokinesia. Thus, whilst Δ^9 -THC produced these effects only in the wild-type mice, anandamide was essentially as potent and effective in producing these effects in CB₁^{-/-} as in CB₁^{+/+} C57BL/6 mice (Di Marzo et al. 2000). Indeed, this putative new receptor may well prove to be a novel target for anti-spasticity and analgesic drugs (Brooks et al. 2002). The presence of specific binding sites for [³H]SR141716A on CB₁^{-/-} C57BL/6 mouse brain membranes may explain the ability of SR141716A both to inhibit [³⁵S]GTP γ S binding to such membranes (Breivogel et al. 2001) and to reduce milk intake and survival of newborn CB₁^{-/-} C57BL/6 mice (Fride et al. 2003).

Central TRPV1-Like Receptors

Evidence has emerged for the presence of G protein-coupled, non-CB₁ receptors on glutamatergic axonal terminals in the hippocampus with which at least some cannabinoid receptor agonists can interact to inhibit glutamate release. More specifically, results from electrophysiological experiments with hippocampal slices

obtained from rats or $CB_1^{+/+}$ CD1 mice have shown that *R*-(+)-WIN55212 reduces both excitatory postsynaptic currents (EPSCs) evoked in CA1 pyramidal cells or dentate granule cells and paired pulse facilitation of EPSCs, even though it has not proved possible to detect CB_1 receptor immunostaining on axonal terminals that form glutamatergic synapses in rat hippocampus (Hájos and Freund 2002a; Hájos et al. 2000, 2001). Similar results have been obtained in experiments with $CB_1^{-/-}$ CD1 mouse hippocampal slices (Hájos et al. 2001). *R*-(+)-WIN55212 also inhibits potassium-evoked glutamate release from hippocampal synaptosomes obtained from rats or from $CB_1^{+/+}$ or $CB_1^{-/-}$ mice in an SR141716A- and AM251-independent manner (Köfalvi et al. 2003). Evidence for an involvement of G proteins in the apparent inhibitory effect of *R*-(+)-WIN55212 on glutamate release in mouse hippocampal slices comes from the finding that this effect is pertussis toxin-sensitive (Misner and Sullivan 1999).

The ability of *R*-(+)-WIN55212 to reduce evoked EPSCs in rat hippocampal slices is shared by CP55940 and capsaicin, and all three of these agonists are antagonized by the TRPV1 receptor antagonist capsazepine (Hájos and Freund 2002a). Because the peripheral TRPV1 receptor is neither activated by *R*-(+)-WIN55212 or CP55940 nor coupled to G proteins, it may be that *R*-(+)-WIN55212, CP55940 and capsaicin modulate central glutamate release by acting through a novel metabotropic “vanilloid-like” receptor. Consequently, it would be of interest to establish first whether capsaicin enhances $GTP\gamma S$ binding to brain membranes, and secondly whether *R*-(+)-WIN55212-induced enhancement of $GTP\gamma S$ binding to $CB_1^{-/-}$ mouse brain membranes (see above) can be antagonized by capsazepine.

Evidence for the presence of vanilloid-like receptors in the hippocampus has also been obtained by Al-Hayani et al. (2001). They found paired-pulse depression in the CA1 region of rat hippocampal slices to be increased both by anandamide and by two other TRPV1 receptor agonists, capsaicin and resiniferatoxin, in a manner that was sensitive to antagonism by capsazepine but not by the CB_1 receptor antagonist AM281. Given the results obtained by Hájos et al. (see above), it is possible that these agonists were acting through central vanilloid-like receptors to cause a decrease in excitatory glutamatergic transmission. Alternatively, they may have been acting through these putative receptors to cause an increase in inhibitory γ -aminobutyric acid (GABA)ergic transmission. If anandamide was acting through vanilloid-like receptors, then it apparently activates them more readily than CB_1 receptors, which contrasts with reports that this endocannabinoid interacts less potently with established TRPV1 receptors than with CB_1 receptors (Sect. 4.1.1). In contrast to anandamide, both *R*-(+)-WIN55212 and 2-arachidonoyl glycerol were found to decrease paired-pulse depression in an SR141716A or AM281-sensitive manner (Al-Hayani et al. 2001; Paton et al. 1998). This would suggest that unlike anandamide, these two agonists interact preferentially with CB_1 receptors in this experimental model. There is evidence that anandamide and/or capsaicin can modulate glutamatergic transmission in brain areas other than the hippocampus in a manner that is CB_1 -independent and susceptible to antagonism by capsazepine and/or iodoresiniferatoxin. These brain areas include rat locus coeruleus, substantia nigra and medullary dorsal horn (Jennings et al. 2003; Marinelli et al. 2002,

2003). In these experiments, however, glutamatergic transmission was facilitated by anandamide and/or capsaicin.

There is currently no support for the hypothesis that *R*-(+)-WIN55212 inhibits glutamate release in the hippocampus by acting on the non-CB₁, non-CB₂ molecular target that is thought to mediate its enhancement of GTPγS binding to central neuronal membranes (see above). Thus, although *R*-(+)-WIN55212 does suppress evoked EPSCs and paired pulse facilitation in CB₁^{-/-} CD1 mouse hippocampal slices (Hájos et al. 2001), it does not enhance GTPγS binding to CB₁^{-/-} CD1 mouse hippocampal membranes (Monory et al. 2002). Also, whilst CP55940 suppresses evoked EPSCs in rat hippocampal slices (Hájos and Freund 2002a) and potassium-evoked glutamate release from rat hippocampal synaptosomes (Köfalvi et al. 2003), it does not share the ability of *R*-(+)-WIN55212 or anandamide to enhance GTPγS binding to CB₁^{-/-} C57BL/6 mouse brain membranes (Breivogel et al. 2001).

Peripheral Nervous System

Results from experiments with phenylephrine-precontracted rat isolated mesenteric and hepatic arteries suggest that Δ⁹-THC can relax these vessels by acting on capsaicin-sensitive perivascular sensory neurons to induce release of calcitonin gene-related peptide (Zygmunt et al. 2002). The underlying mechanism is most probably CB₁ and CB₂ receptor-independent, as this relaxant effect of Δ⁹-THC was not prevented by 300 nM SR141716A or by 30 nM AM251 and as the CB₁/CB₂ receptor agonists HU-210 and CP55940 lacked detectable relaxant activity, whereas cannabinalol, which has relatively low activity as a cannabinoid receptor agonist (Sect. 3.1), was equipotent with Δ⁹-THC. The possibility, that Δ⁹-THC was acting through ionotropic or metabotropic glutamate receptors was also excluded. Other observations made in this investigation were that Δ⁹-THC- and cannabinalol-induced activation of CGRP release from rat arterial segments could be prevented by capsaicin pretreatment and that Δ⁹-THC- and cannabinalol-induced relaxations of precontracted arterial segments could be attenuated by the noncompetitive TRPV1 antagonist ruthenium red. However, these cannabinoids were most probably not acting through TRPV1 receptors in these experiments. Thus, the competitive TRPV1 antagonist capsazepine did not attenuate the vasorelaxant effects of Δ⁹-THC and cannabinalol, and in contrast to both capsaicin and anandamide, Δ⁹-THC also relaxed phenylephrine-precontracted mesenteric arterial segments that had been obtained from TRPV1^{-/-} mice. In more recent experiments, Jordt et al. (2004) have obtained evidence that Δ⁹-THC and cannabinalol may have induced vasorelaxation by acting through ANKTM1, another member of the transient receptor potential (TRP) family of ion channels that, unlike the TRPV1 receptor, appears to be insensitive to anandamide and is implicated in the detection of noxious cold. ANKTM1 was found to be insensitive to HU-210, CP55940 and 2-arachidonoyl glycerol.

It has also been proposed that the terminals of sympathetic neurons supplying cardiovascular tissue express a non-I₁, non-I₂ subtype of the putative imidazoline receptor that is both CB₁ receptor-like and α₂-adrenoceptor-like and that mediates inhibition of evoked noradrenaline release when activated (reviewed in

Göthert et al. 1999; Molderings and Göthert 1999; Pertwee 2004a). There is evidence that this putative receptor can be activated both by the cannabinoids—CP55940, *R*-(+)-WIN55212 and anandamide—and by non-CB₁, non-CB₂ ligands such as aganodine and clonidine, and that this activation is sensitive to antagonism by SR141716A (1 μM), LY320135 (0.1 or 1 μM) and rauwolscine (30 μM) (reviewed in Pertwee 2004a). It also appears that this proposed receptor may belong to the G protein-coupled receptor family originally known as endothelial differentiation gene (EDG) receptors and that it can be activated by 1-oleoyl-lysophosphatidic acid (Molderings et al. 2002).

Mang et al. (2001) have obtained evidence that anandamide can act on nerve terminals of the myenteric plexus–longitudinal muscle preparation of the guinea-pig ileum to inhibit electrically evoked release of the contractile transmitter acetylcholine through a mechanism that is independent of both TRPV1 and CB₁ receptors. Thus, the inhibitory effects of anandamide on electrically evoked release of [³H]acetylcholine and on electrically evoked contractions of this isolated tissue preparation were insensitive to antagonism by 1 μM capsazepine. They were also much less sensitive to antagonism by SR141716A than expected for CB₁-mediated effects. Results from other experiments with this tissue preparation suggest that anandamide can increase both basal acetylcholine release from neurons and longitudinal muscle tone by acting on neuronal TRPV1 receptors (Mang et al. 2001). Additional support for the presence of a non-CB₁ receptor for anandamide in the gastro-intestinal tract comes from experiments both with the strips of longitudinal muscle obtained from guinea-pig distal colon (Kojima et al. 2002) and with the rat isolated gastric fundus (Storr et al. 2002). In the colon experiments, evidence was obtained that anandamide, possibly after its conversion to active metabolites, can induce contractions by acting through a TRPV1 and CB₁ receptor-independent mechanism (Kojima et al. 2002). 2-Arachidonoyl glycerol also seems to act through such a mechanism to induce contractions of this tissue preparation (Kojima et al. 2002). In the gastric fundus experiments it was found that at 10 μM, the CB₂-selective antagonist AM630 attenuated anandamide- but not *R*-(+)-WIN55212-induced inhibition of electrically evoked contractions (Storr et al. 2002). It is likely that anandamide was acting on prejunctional neurons in this tissue, as it did not affect contractions produced by 5-HT or carbachol. AM630 has also been found to antagonize Δ⁹-THC, CP55940, *R*-(+)-WIN55212, methanandamide and anandamide in the mouse isolated vas deferens in an agonist-dependent and competitive manner. However, in this bioassay system, AM630 was less potent as an antagonist of anandamide than of *R*-(+)-WIN55212 (Pertwee et al. 1995). In view of evidence that the mouse vas deferens expresses neuronal CB₂-like receptors that can mediate inhibition of electrically evoked contractions (Griffin et al. 1997; Sect. 4.1.3), it may be that AM630 was producing its antagonism of cannabinoids in this tissue by competing for these putative CB₂-like receptors.

4.1.5

Receptors for Abnormal-Cannabidiol

Cardiovascular System

There is evidence, mainly from *in vitro* experiments with rat or mouse phenylephrine- or methoxamine-precontracted buffer-perfused isolated mesenteric arterial beds or isolated mesenteric arterial segments, for the presence in these tissues of non-CB₁, non-CB₂ receptors with which anandamide and methanandamide can interact to induce a relaxant effect (reviewed in Howlett et al. 2002; Pertwee 2004a; Wiley and Martin 2002). There are several reasons for believing that these are not CB₁ or CB₂ receptors. First, relaxation is not induced in rat precontracted mesenteric arterial beds by 2-arachidonoyl glycerol or by established non-eicosanoid cannabinoid receptor agonists such as Δ^9 -THC or *R*-(+)-WIN55212 (Wagner et al. 1999) but is induced in rat and mouse precontracted mesenteric arterial beds or rat precontracted mesenteric arterial segments by two cannabidiol analogues, abnormal-cannabidiol and O-1602 (Fig. 12), neither of which exhibits significant affinity for CB₁ receptors (Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003; Showalter et al. 1996). Second, anandamide, methanandamide and abnormal-cannabidiol also relax precontracted buffer-perfused mesenteric arterial beds of CB₁^{-/-} knockout or CB₁^{-/-}/CB₂^{-/-} double-knockout C57BL6J mice (Járai et al. 1999). Third, the CB₁-selective antagonist AM281 (1 μ M) and the CB₂-selective antagonist AM630 (10 μ M) do not attenuate abnormal-cannabidiol-induced relaxations of rat precontracted mesenteric arterial segments (Ho and Hiley 2003). Although SR141716A has been found to oppose the vasorelaxant effects of abnormal-cannabidiol, methanandamide and anandamide in rat or mouse precontracted mesenteric arterial beds or segments, this is generally with a potency lower than expected from its affinity for CB₁ receptors (Ho and Hiley 2003; Járai et al. 1999). Negative results obtained with capsaicin and capsazepine also make it unlikely that the putative “abnormal-cannabidiol” receptor is a TRPV1 receptor (Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003).

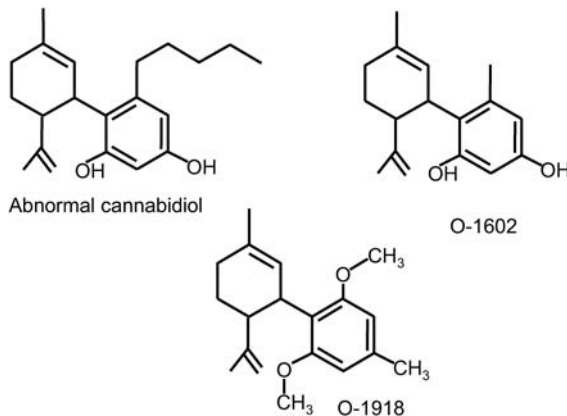


Fig. 12. The structures of abnormal cannabidiol, O-1602 and O-1918

One cannabidiol analogue has been found to behave as a selective abnormal-cannabidiol receptor antagonist. This is O-1918 (Fig. 12), which lacks detectable affinity for CB₁ and CB₂ receptors and, at concentrations of 1 to 30 μM, opposes abnormal-cannabidiol and anandamide-induced relaxations of rat arterial segments and does not reduce vasomotor tone when administered alone (Offertáler et al. 2003). It has also been found to attenuate abnormal-cannabidiol-induced hypotension in anaesthetized mice at doses not affecting hypotension induced by the CB₁/CB₂ receptor agonist HU -210 (Offertáler et al. 2003). Cannabidiol also behaves as a selective abnormal-cannabidiol receptor antagonist in both the rat mesenteric arterial bed and the anaesthetized mouse (Járai et al. 1999). However, in contrast to O-1918, it has been found to share the ability of abnormal-cannabidiol to relax rat precontracted mesenteric arterial segments (Offertáler et al. 2003).

It is likely that there are two sub-types of abnormal-cannabidiol-sensitive receptor in mesenteric arteries capable of mediating a relaxant effect, one expressed by endothelial cells and the second by non-endothelial cells (reviewed in Pertwee 2004a). Activation of the endothelial receptor appears to open large conductance calcium-activated potassium (BK_{Ca}) channels, whereas the non-endothelial receptor seems to signal mainly through inhibition of L-type calcium channels (Begg et al. 2003; Ho and Hiley 2003; Járai et al. 1999; Offertáler et al. 2003). There is also now evidence that abnormal-cannabidiol receptors can mediate stimulation of the migration of vascular endothelial cells through a mechanism that is G_{i/o} protein-coupled and susceptible to antagonism by O-1918 (Mo et al. 2004).

Microglial Cells

Experiments with the mouse microglial cell line BV-2 (Walter et al. 2003) have provided evidence that microglial cells express receptors that have certain properties in common with the putative vascular abnormal-cannabidiol receptor discussed above. These include susceptibility to activation by abnormal-cannabidiol and anandamide and to blockade by O-1918 and lack of sensitivity to activation by Δ⁹-THC, at least at concentrations below 3 μM. When activated, these proposed abnormal-cannabidiol-sensitive receptors appear to trigger chemokinetic and chemotactic migration of microglial cells. Such migration can also be induced by 2-arachidonoyl glycerol (EC₅₀=25 nM). This endocannabinoid seems to act through both microglial CB₂ receptors and microglial abnormal-cannabidiol-sensitive receptors, since it is antagonized by cannabidiol at 300 nM and by SR144528 at 30 nM but not by 30 nM SR141716A (Walter et al. 2003). Indeed, it has been proposed that microglial CB₂ receptors and abnormal-cannabidiol receptors interact in a synergistic manner when triggering the migration of microglial cells (Walter et al. 2003). This could explain why the CB₁-selective agonist ACPA (Sect. 3.1), induces microglial cell migration at concentrations well below those at which it has been reported to bind to CB₂ receptors, as this compound appears to induce migration by acting on both abnormal-cannabidiol-sensitive receptors and CB₂ receptors (Franklin and Stella 2003). By itself, cannabidiol behaves as a weak partial agonist, producing a slight enhancement of basal migration (EC₅₀=250 nM) (Walter et al. 2003). Microglial cells are thought to migrate towards

neuroinflammatory lesion sites and to release proinflammatory cytokines and cytotoxic agents at these sites. Consequently, since Walter et al. (2003) also obtained evidence that the production of 2-arachidonoyl glycerol by microglial cells can be increased by a pathological stimulus, it may be that a CB₂ receptor antagonist and/or an antagonist of the putative abnormal-cannabidiol receptor could come to play a part in the clinical management of neuroinflammation. More recently, evidence has emerged that BV-2 microglial cells express non-CB₁, non-CB₂, non-CB₂-like, non-TRPV1, non-abnormal-cannabidiol G_i/G_o-coupled-receptors upon which the endogenous fatty acid amide palmitoylethanolamide can act at concentrations in the low nanomolar range to potentiate anandamide- but not 2-arachidonoyl glycerol-induced migration of these cells (Franklin et al. 2003). There is also evidence for the presence in rat microglial cells of non-CB₁, non-CB₂, pertussis toxin-insensitive receptors with which *R*-(+)- but not *S*-(-)-WIN55212 can interact to inhibit lipopolysaccharide-induced release of the proinflammatory cytokine tumour necrosis factor- α (Facchinetti et al. 2003).

Mouse Vas Deferens

A finding that abnormal-cannabidiol and cannabidiol can attenuate phenylephrine-induced contractions of the mouse isolated vas deferens points to the presence of abnormal-cannabidiol-sensitive receptors in the smooth muscle cells of this tissue (Pertwee et al. 2002; Thomas et al. 2004). Cannabidiol also decreases methoxamine and noradrenaline-induced contractions of the mouse vas deferens and antagonizes phenylephrine and noradrenaline in an insurmountable manner (Pertwee et al. 2002). It may be, therefore, that cannabidiol, and possibly also abnormal-cannabidiol, are negative allosteric modulators of the α_1 -adrenoceptor.

4.2 Allosteric Sites

There is evidence for the presence of allosteric sites for anandamide and/or certain other cannabinoids on several non-cannabinoid receptors (reviewed in Pertwee 2004a). These are 5-HT₂ receptors (Cheer et al. 1999), 5-HT₃ receptors (Barann et al. 2002; Fan 1995; Godlewski et al. 2003; Oz et al. 2002), α_1 -adrenoceptors (Sect. 4.1.5), M₁ and M₄ muscarinic receptors (Christopoulos and Wilson 2001) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) GLU_{A1} and GLU_{A3} glutamate receptors (Akinshola et al. 1999a,b). The functional consequences of occupation of the proposed allosteric sites on 5-HT₂ receptors (by HU-210) and on M₁ and M₄ receptors (by anandamide, methanandamide and SR141716A) have yet to be determined. However, cannabinoids have been found to inhibit currents triggered by the activation of GLU_{A1} and GLU_{A3} receptors (anandamide) or 5-HT₃ receptors (Δ^9 -THC, *R*-(+)-WIN55212, anandamide, JWH-015 (Fig. 7), CP55940 and the CB₁ receptor antagonist, LY320135). Cannabinoids have also been found to attenuate the von Bezold-Jarisch reflex induced in urethane-anaesthetized rats by 5-HT₃ receptor activation (CP55940 and *R*-(+)-WIN55212) and to oppose α_1 -

adrenoceptor-mediated contractions of the mouse vas deferens (cannabidiol). In addition, there are reports that 2-arachidonoyl glycerol and 5-HT each binds more readily to washed human platelets in the presence of the other compound (Maccarrone et al. 2003) and that 5-HT enhances binding of *R*-(+)-WIN55212 to CB₁ receptors (Devlin and Christopoulos 2002). Importantly, cannabinoids inhibited 5-HT₃ receptor currents in transfected human embryonic kidney cells with a rank order of potency, Δ^9 -THC > *R*-(+)-WIN55212 > anandamide > JWH-015 > LY320135 > CP55940 (Barann et al. 2002), that does not correlate with their CB₁ or CB₂ receptor affinities or intrinsic activities (Sect. 3). The IC₅₀ values of these ligands were 38, 104, 130, 147, 523 and 648 nM, respectively (Barann et al. 2002). In contrast, the IC₅₀ values of anandamide for inhibition of kainate-activated currents in GLU_{A1}- and GLU_{A3}-transfected *Xenopus laevis* oocytes exceeded 100 μ M (Akinshola et al. 1999b). In addition, some cannabinoids, including anandamide, methanandamide, *R*-(+)-WIN55212, Δ^9 -THC and cannabidiol, may serve as negative modulators of delayed rectifier potassium channels (reviewed in Pertwee 2004a). There is also evidence that nanomolar concentrations of anandamide can block low-voltage-activated (T-type) calcium channels through a mechanism that is independent of CB₁ and CB₂ receptors and of G proteins (Chemin et al. 2001). Evidence has also recently emerged for the presence of an allosteric site on the cannabinoid CB₁ receptor (R. Pertwee, R. Ross and M. Price, unpublished).

4.3

Some CB₁- and CB₂-Independent Actions of Cannabidiol, HU-211 and Other Phenol-Containing Cannabinoids

4.3.1

Neuroprotective Actions

Cannabinoids that contain a phenol group possess anti-oxidant (electron donor) activity that is sufficient to protect neurons against oxidative stress associated, for example, with glutamate-induced excitotoxicity. Thus, as discussed in greater detail elsewhere (El-Remessy et al. 2003; Fowler 2003; Hampson et al. 1998, 2000; Marsicano et al. 2002; Mechoulam et al. 2002; Pertwee 2004b; Platt and Drysdale 2004; van der Stelt et al. 2002), this anti-oxidant activity is apparently independent of CB₁ or CB₂ receptors as it is exhibited both by the CB₁/CB₂ agonists Δ^9 -THC, HU-210 and CP55940, and by the non-psychoactive phytocannabinoid cannabidiol (Fig. 1) and the *cis* (6a*S*, 10a*S*) enantiomer of 11-hydroxy- Δ^8 -THC-dimethylheptyl, HU-211 (Fig. 4), neither of which has significant affinity for CB₁ or CB₂ receptors (Table 4). Moreover, neurons of CB₁^{-/-} mice are no less well protected from oxidative stress by phenolic cannabinoids than neurons of CB₁^{+/+} mice (Marsicano et al. 2002). The neuroprotective properties of HU-211 are also thought to stem from its ability to behave as a non-competitive antagonist at *N*-methyl-D-aspartate (NMDA) receptors and to inhibit tumour necrosis factor- α production (Mechoulam et al. 2002; Darlington 2003), and it is possible that cannabidiol may also protect from glutamate-induced excitotoxicity by opposing

metabotropic glutamate receptor-mediated release of calcium from intracellular stores (Drysdale et al. 2004). Non-phenolic cannabinoids have been reported to lack anti-oxidant activity (Marsicano et al. 2002). Even so, some non-phenolic (and phenolic) cannabinoids can protect against glutamate-induced excitotoxicity by acting through receptors to inhibit neuronal glutamate release (possibly putative TRPV1-like receptors; Sect. 4.1.4) and calcium entry into neurons through N- and P/Q-type channels (CB₁ receptors) (Fowler 2003; Mechoulam et al. 2002; van der Stelt et al. 2002).

4.3.2

Other Actions of Cannabidiol

Results from in vitro experiments suggest that cannabidiol has a number of CB₁/CB₂ receptor-independent actions through which it may affect neurotransmission (reviewed in Pertwee 1988, 2004b). For example, there is evidence that at concentrations in the nanomolar or low micromolar range, this cannabinoid enhances spontaneous or evoked release of certain transmitters, antagonizes R-(+)-WIN55212- and CP55940-induced inhibition of electrically evoked contractile transmitter release in the mouse isolated vas deferens through a CB₁-independent mechanism and inhibits the uptake of calcium, 5-HT, noradrenaline and dopamine by rat or mouse synaptosomes. Higher concentrations of cannabidiol inhibit anandamide uptake by rat basophilic leukaemia cells, the metabolism of this endocannabinoid by fatty acid amide hydrolase and the synaptosomal uptake of GABA. There is also evidence that cannabidiol is a TRPV1 receptor agonist, a ligand for the putative abnormal-cannabidiol receptor (Sect. 4.1.5) and a negative allosteric modulator of α_1 -adrenoceptors (Sect. 4.1.5) and delayed rectifier potassium channels (Sect. 4.2). In addition, cannabidiol inhibits/induces certain cytochrome P450 (CYP450) enzymes, has anti-tumour activity and possesses anti-inflammatory properties that may be due at least in part to inhibition of lipoxygenase activity and cytokine release (Pertwee 2004b).

The CB₁ and CB₂ affinities of cannabidiol can be greatly enhanced both by changing its stereochemistry from (-)-(3R, 4R) to (+)-(3S, 4S) and by making certain structural modifications (reviewed in Howlett et al. 2002; Pertwee 2004b). Cannabidiol analogues with particularly high affinities for CB₁ and CB₂ receptors are (+)-(3S, 4S)-4'-dimethylheptyl-cannabidiol and (+)-(3S, 4S)-7-hydroxy-4'-dimethylheptyl-cannabidiol (Bisogno et al. 2001). Several (-)-(3R, 4R)-analogues of cannabidiol with high CB₁ and CB₂ affinities have also been developed, for example O-1660, O-1871 and O-1422 (Wiley et al. 2002). Whether these (+)-(3S, 4S)- and (-)-(3R, 4R)-analogues of cannabidiol are agonists or antagonists remains to be established. However, one (-)-(3R, 4R)-cannabidiol analogue that is already known to be a potent CB₂-selective agonist is HU-308 (Sect. 3.1), whilst another cannabidiol analogue, O-2654, behaves as a reasonably potent CB₁ receptor antagonist (Sect. 3.4).

Finally, there is evidence that cannabidiol can induce apoptosis in cultures of at least some types of human cancer cell: HL-60 myeloblastic leukaemia cells and

glioma cells. More specifically, it has been reported to produce signs of apoptosis at 3.2 μM in γ -irradiated HL-60 cells, at 12.7 μM in non-irradiated HL-60 cells and at 25 μM but not 10 μM in U87 and U373 glioma cells (Gallily et al. 2003; Massi et al. 2004). At these or higher concentrations, cannabidiol did not induce detectable apoptosis in γ -irradiated or non-irradiated monocytes obtained from normal individuals (Gallily et al. 2003).

5

CB₁ Receptor Oligomerization

There is some evidence that the CB₁ receptor can exist as a homodimer and also that it may form heterodimers or oligomers with one or more other classes of co-expressed G protein-coupled receptor (e.g. dopamine D₂ and opioid receptors) (Wager-Miller et al. 2002). Resulting cross-talk between CB₁ and non CB₁ receptors may involve the sequestration of G proteins either from other receptor types by CB₁ receptors (reviewed in Pertwee 2003) or conversely, from CB₁ receptors by other receptor types. For example, results obtained from experiments with primary cultures of rat striatal neurons (Glass and Felder 1997) and with human embryonic kidney cells co-transfected with CB₁ and dopamine D₂ receptors (Jarrahian et al. 2004) suggest that D₂ receptors can sequester $G\alpha_{i/o}$, so as to cause co-expressed CB₁ receptors to switch coupling from $G\alpha_{i/o}$ to $G\alpha_s$. Interestingly, Jarrahian et al. (2004) also found that in the human embryonic kidney cells expressing both CB₁ and D₂ receptors, persistent activation of the D₂ receptors promoted the re-establishment of CB₁ receptor coupling with $G\alpha_{i/o}$. Results from other in vitro experiments have provided evidence that in the presence of ongoing $G\alpha_s$ -mediated adenylate cyclase stimulation by adenosine A₂ receptor activation, D₂ and CB₁ receptor agonists can interact synergistically through their respective receptors to produce further adenylate cyclase stimulation via $\beta\gamma$ -subunits released from $G\alpha_{i/o}$ (Yao et al. 2003).

6

Future Directions

Clearly there is now incontrovertible evidence for the existence of a mammalian endocannabinoid system that consists of at least two types of cannabinoid receptor, CB₁ and CB₂, and of endogenous agonists (endocannabinoids) for these receptors. Agonists that activate both these receptor types with similar potency or that show marked selectivity for one or other receptor type have been discovered, as have potent CB₁- and CB₂-selective cannabinoid receptor antagonists. Quantitative and sensitive in vitro and in vivo bioassays for these ligands are also available, and these have played a crucial role in determining the CB₁ and CB₂ receptor affinities and intrinsic activities of a number of cannabinoids. There is good evidence that the endocannabinoid system can become tonically active and that this is due in some instances to endocannabinoid release and in other instances to the ability of cannabinoid receptors to exist in a constitutively active state, not only when over-

expressed in cultured cells but also when expressed naturally. The existence of such constitutive activity is reflected in the pharmacological properties of established cannabinoid receptor antagonists, all of which appear to be inverse agonists rather than neutral antagonists. Ligands that behave as neutral cannabinoid receptor antagonists are beginning to be described in the literature. These now need to be characterized more fully, as such antagonists would serve as important additional pharmacological tools and might also possess advantages over inverse agonists in the clinic. Evidence for the presence of non-CB₁, non-CB₂ pharmacological targets for at least some cannabinoid receptor agonists is emerging, prompting a need to establish the extent to which these proposed additional targets contribute to the pharmacology of these agonists. For some of these targets, ligands that do not also interact with CB₁ or CB₂ receptors have already been identified, and it will now be important to characterize the actions of these ligands more fully and to investigate the possibility of developing potent and selective non-CB₁, non-CB₂ agonists for all the proposed new targets. This in turn will greatly facilitate a fuller understanding of these targets as well as the discovery of any additional targets. The extent to which cross-talk can occur between identical (e.g. CB₁-CB₁) or different pharmacological targets for cannabinoids (e.g. between CB₂ and abnormal cannabidiol receptors), or between cannabinoid and non-cannabinoid targets (e.g. between CB₁ and dopamine D₂ receptors), and the nature of the mechanisms that underlie such cross-talk also merit further investigation.

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