Minireview

Cannabinoid signalling

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Abstract

After their discovery, the two known cannabinoid receptors, CB1 and CB2, have been the focus of research into the cellular signalling mechanisms of cannabinoids. The initial assessment, mainly derived from expression studies, was that cannabinoids, via Gα proteins, negatively modulate cyclic AMP levels, and activate inward rectifying K+ channels. Recent findings have complicated this assessment on different levels: (1) cannabinoids include a wide range of compounds with varying profiles of affinity and efficacy at the known CB receptors, and these profiles do not necessarily match their biological activity; (2) CB receptors appear to be intrinsically active and possibly coupled to more than one type of G protein; (3) CB receptor signalling mechanisms are diverse and dependent on the system studied; (4) cannabinoids have other targets than CB receptors. The aim of this mini review is to discuss the current literature regarding CB receptor signalling pathways. These include regulation of adenylyl cyclase, MAP kinase, intracellular Ca2+, and ion channels. In addition, actions of cannabinoids that are not mediated by CB1 or CB2 receptors are discussed.

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Keywords: Cannabinoid receptor; Signal transduction; Cannabinoids; G proteins; Second messengers; Ion channels

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Introduction

Cannabinoids can be broadly defined as compounds with actions on cannabinoid receptors together with chemically related compounds. They include the plant Cannabis sativa-derived compounds with Δ⁹-THC (see Table 1 for a list of abbreviations) as prototype, and the related group of synthetic drugs typified by the Pfizer compound CP 55,940. In addition, there are the aminoalkyl indoles developed by Winthrop (e.g. WIN55,212-2), and finally the endogenous eicosanoids with anandamide as the compound that is the most widely studied.

The picture that has emerged with regard to the biological actions of these classes of compounds is complex. Initially it seemed that cannabinoids act, with various degrees of affinity and efficacy, on identified specific receptors which belong to the 7-transmembrane region receptor family and are G<sub>i/o</sub> protein coupled to adenylyl cyclase (negatively) and inward rectifying potassium channels (positively). However, numerous studies have now shown that the signalling pathways utilised by cannabinoid receptors are a great deal more diverse than originally established, and that some cannabinoids have activity that is not mediated by the known cannabinoid receptors.

In this review the current knowledge of cannabinoid receptor signalling is discussed, together with the evidence for other cellular targets for cannabinoids.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>List of abbreviations</th>
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<tbody>
<tr>
<td>2-AG</td>
<td>2-arachidonoyl glycerol</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>abn-CBD</td>
<td>abnormal cannabidiol</td>
</tr>
<tr>
<td>AC</td>
<td>adenylate cyclase</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BTX</td>
<td>botulinus toxin</td>
</tr>
<tr>
<td>CAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CB receptor</td>
<td>cannabinoid receptor</td>
</tr>
<tr>
<td>D receptor</td>
<td>dopamine receptor</td>
</tr>
<tr>
<td>DRG</td>
<td>dorsal root ganglion</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GDP</td>
<td>guanylate diphosphate</td>
</tr>
<tr>
<td>GIRK channel</td>
<td>G-protein-coupled inwardly rectifying K⁺ channel</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein-coupled receptor</td>
</tr>
<tr>
<td>GTP</td>
<td>guanylate triphosphate</td>
</tr>
<tr>
<td>I&lt;sub&gt;A&lt;/sub&gt;</td>
<td>A-type K⁺ current</td>
</tr>
<tr>
<td>I&lt;sub&gt;D&lt;/sub&gt;</td>
<td>D-type K⁺ current</td>
</tr>
<tr>
<td>I&lt;sub&gt;M&lt;/sub&gt;</td>
<td>M-type K⁺ current</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>InsP&lt;sub&gt;3&lt;/sub&gt;</td>
<td>inositol 1,4,5-trisphosphate</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MEK</td>
<td>mitogen activated kinase</td>
</tr>
<tr>
<td>NACHr</td>
<td>nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>P3K</td>
<td>phosphatidylinositol-3-kinase</td>
</tr>
<tr>
<td>PKA/C</td>
<td>protein kinase A/C</td>
</tr>
<tr>
<td>PLAA/C</td>
<td>phospholipase A/C</td>
</tr>
<tr>
<td>PTX</td>
<td>pertussis toxin</td>
</tr>
<tr>
<td>TASK channel</td>
<td>TWIK-related acid-sensitive K⁺ channel</td>
</tr>
<tr>
<td>TRPV1 receptor</td>
<td>transient receptor potential vanilloid-1 receptor</td>
</tr>
<tr>
<td>VOCC</td>
<td>voltage-operated Ca&lt;sup&gt;2+&lt;/sup&gt; channel</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>Δ⁹-tetrahydrocannabinol</td>
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Cannabinoid receptors

The resin secreted by the Asian hemp C. sativa contains about 60 active compounds of which Δ⁹-THC is the principal psychoactive component (Gaoni and Mechoulam, 1964). Due to its highly lipophilic nature it was initially believed that Δ⁹-THC exerted its effects by interacting with the plasma membrane, stimulating or inhibiting membrane-associated enzymes and altering the physical state of ion channels (Hillard et al., 1985; Martin, 1986). Speculation about the cellular actions of cannabinoids was finally resolved when functional inhibition of adenylyl cyclase, and hence a reduction in cAMP, the second messenger produced by adenylyl cyclase, was observed following the addition of Δ⁹-THC to neuroblastoma cells (Howlett and Fleming, 1984). The inhibition was blocked with PTX suggesting the involvement of a G<sub>i/o</sub> protein (Howlett et al., 1986). These actions, as well as studies demonstrating stereoselectivity of the (−)-enantiomers of Δ⁹-THC (Dewey, 1986) and specific binding of radiolabeled agonists in rat brain membranes (Devane et al., 1988), indicated that at least most of the central cannabinoid effects were mediated by a specific membrane receptor protein, the CB<sub>1</sub> receptor. The CB<sub>1</sub> receptor was eventually cloned from rat cerebral cortex (Matsuda et al., 1990) and later from human brain and testis (Gerard et al., 1991), and mouse brain (Chakrabarti et al., 1995). A splice variant of the CB<sub>1</sub> receptor was also isolated from human lung and was designated CB<sub>1A</sub> (Shire et al., 1995).

The CB<sub>1</sub> receptor is expressed in high abundance within certain regions of the brain that correlate well with the observed effects of cannabinoids, including impairments in cognition, memory, learning and motor coordination (Abood and Martin, 1992). Hence, CB<sub>1</sub> receptors have been isolated in the hippocampus, basal ganglia (striatum, substantia nigra, globus pallidus), cerebral cortex, amygdala and cerebellum (Herkenham et al., 1990; Glass et al., 1997; Tsou et al., 1998). Peripherally, CB<sub>1</sub> receptors have been identified in the spleen and tonsils (Galiegue et al., 1995), the guinea-pig small intestine (Pertwee et al., 1996a), the mouse urinary bladder (Pertwee and Fernando, 1996), the mouse vas deferens (Pertwee et al., 1996b), sympathetic nerve terminals (Ishac et al., 1996; Vizi et al., 2001), hamster smooth muscle cells (Filipeanu et al., 1997), cat vascular smooth muscle cells (Gebremedhin et al., 1999) and at very low levels in adrenal gland, heart, prostate, uterus and ovary (Galiegue et al., 1995).

Recent studies used in vivo imaging techniques (positron emission tomography) to identify CB<sub>1</sub> receptor occupancy in mouse brain. The technique requires the use of radioisotopes that specifically bind to the target receptor. Interestingly, concentrations of cannabinoids that produced a profound sedation and inhibition of locomotor activity in mice did not reduce CB<sub>1</sub> receptor binding by radioisotopes in the cerebellum and hippocampus (Gifford et al., 2002). This suggests that the occupancy of the CB<sub>1</sub> receptor necessary for the behavioural effects of cannabinoids is very low. Detailed pharmacology and the interesting intrinsic properties of CB<sub>1</sub> receptors have just been reviewed by Pertwee (2005) in this journal in the context of antagonism.
The second cannabinoid receptor, the CB₂ receptor, was cloned from human promyelocytic leukaemia cells (HL-60 cells) (Munro et al., 1993). This receptor shares with the CB₁ receptor the structural feature typified by seven transmembrane spanning domains and is also coupled to a PTX-sensitive G protein. The clone has 68% amino acid sequence homology to the CB₁ receptor within the transmembrane domains, and only 44% homology throughout the total protein (Munro et al., 1993). CB₂ receptors are restricted to the periphery where they have been observed in the marginal zone of the spleen (Munro et al., 1993, Schatz et al., 1997), in tonsils and on immune cells (B-cells, monocytes, T-cells) (Munro et al., 1993, Galiegue et al., 1995, Schatz et al., 1997). The localisation of CB₂ receptors in immune tissues implies that some cannabinoid-induced immunosuppression involves a receptor-mediated process. The exception to the rule that CB₂ receptors are only found in the periphery is the report that CB₂ receptors can be expressed in brain microglial cells under inflammatory conditions (Nunez et al., 2004).

New data suggests the presence of novel, as yet, uncloned cannabinoid receptors. Using the brains of CB₁ knockout mice (CB₁⁻/⁻) it was shown that there was significant (though reduced) binding of the cannabinoid agonist [³H]WIN 55,212-2 (Breivogel et al., 2001). Moreover, both WIN 55,212-2 and the endogenous cannabinoid anandamide were still able to stimulate some labelled non-hydrolysable GTP ([³⁵S]GTPγS) binding in CB₁⁻/⁻ brain, an indicator of GPCR activation. This effect was not blocked by the CB₁ receptor antagonist SR 141716A. Significant levels of stimulation were observed in the cortex and hippocampus. In accordance with this finding, Monory et al. (2002) showed that WIN 55,212-2 was able to stimulate [³⁵S]GTPγS binding in the cerebellum of CB₁⁻/⁻ mice. SR 141716A could not reverse this effect. Another study also using CB₁⁻/⁻ mice found that WIN 55,212-2 could inhibit excitatory glutamatergic postsynaptic currents in the hippocampus (Hajós et al., 2001). The cannabinoid-mediated inhibition was sensitive to SR 141716A and was also inhibited by the vanilloid TRPV1 receptor antagonist capsazepine (Hajós et al., 2001; Hajós and Freund, 2002a,b). Consistent with these observations WIN 55,212-2 attenuated the release of [³H]glutamate from CB₁⁻/⁻ mouse hippocampal synaptosomes (Kofalvi et al., 2003). However, in contrast to the electrophysiological studies, SR 141716A and capsazepine did not antagonise the effect of WIN 55,212-2. In fact SR 141716A was shown to potenti ate the inhibitory effect of WIN 55,212-2 (Kofalvi et al., 2003). In the basolateral amygdala of anaesthetised rats WIN 55,212-2 inhibited neuronal firing in an SR 141716A- and capsazepine-sensitive manner (Pistis et al., 2004). HU-210, another potent CB₁ agonist, could not mimic the effects of WIN 55,212-2 (Pistis et al., 2004). Another study looking at a cannabinoid-mediated inhibition of cAMP formation in mouse astrocytes reported an SR 141716A-insensitive action of WIN 55,212-2 (Sagan et al., 1999). Immunohistochemical staining confirmed that these astrocytes did not express CB₁ receptors, although the inhibitory actions of WIN 55,212-2 could be blocked by PTX suggesting the involvement of a GPCR (Sagan et al., 1999).

In the periphery the endogenous cannabinoid anandamide was shown to induce mesenteric vasodilatation in CB₁⁻/⁻ mice, which was SR 141716A-sensitive (Jarai et al., 1999). This novel receptor differs from those in the brain as WIN 55,212-2 was not able to produce vasodilatation, and capsazepine was unable to inhibit vasodilatation (Jarai et al., 1999). Instead a non-psychoactive synthetic cannabinoid analogue, abn-CBD, was found to selectively stimulate the endothelial receptor. Recently, activation of this receptor by abn-CBD was also shown to induce human umbilical vein endothelial cell migration (Mo et al., 2004).

Taken together, the work discussed above indicates that cannabinoid effects are clearly not restricted to the known CB receptors and it is expected that in the near future the list of CB receptor-coupled signal transduction targets

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Signalling Target</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB₁</td>
<td>G₁o</td>
<td>+</td>
<td>Bonhaus et al., 1998; Bouaboula et al., 1999</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td>−</td>
<td>Felder et al., 1993; Wade et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Felder et al., 1998; Busch et al., 2004</td>
</tr>
<tr>
<td>p38MAPK</td>
<td></td>
<td>+</td>
<td>Derkinderen et al., 2001; Derkinderen et al., 1995b</td>
</tr>
<tr>
<td>p42/p44 MAPK</td>
<td></td>
<td>+</td>
<td>Derkinderen et al., 2003</td>
</tr>
<tr>
<td>PI3K/PKB</td>
<td></td>
<td>+</td>
<td>Sanchez et al., 1998b; Galve-Roperh et al., 2002</td>
</tr>
<tr>
<td>Ceramide</td>
<td></td>
<td>+</td>
<td>Sanchez et al., 1998a; Galve-Roperh et al., 2000</td>
</tr>
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<td>VOCCs:</td>
<td></td>
<td>−</td>
<td>Pan et al., 1996; Wilson et al., 2001</td>
</tr>
<tr>
<td>N-type</td>
<td></td>
<td>−</td>
<td>Twitchell et al., 1997; Hampson et al., 1998</td>
</tr>
<tr>
<td>L-type</td>
<td></td>
<td>−</td>
<td>Gebremedhin et al., 1999; Straiker et al., 1999</td>
</tr>
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<td>K⁺ channels:</td>
<td></td>
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<td>−</td>
</tr>
<tr>
<td>GIRK</td>
<td></td>
<td></td>
<td>McAllister et al., 1999; Robbe et al., 2001; Hampson et al., 1998</td>
</tr>
<tr>
<td>I₄</td>
<td></td>
<td></td>
<td>Pan et al., 2000</td>
</tr>
<tr>
<td>I₉</td>
<td></td>
<td></td>
<td>Mu et al., 1999; Schweitzer, 2000</td>
</tr>
<tr>
<td>NMDA receptor</td>
<td></td>
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</tr>
<tr>
<td>[Ca²⁺]ᵢ</td>
<td></td>
<td>+</td>
<td>Netzeband et al., 1999; Fimiani et al., 1999; Begg et al., 2001; Demuth et al., 2004</td>
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<tr>
<td>Arachidonic acid</td>
<td></td>
<td></td>
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<tr>
<td>PLC/InsP₃</td>
<td></td>
<td>+</td>
<td>Sugiyama et al., 1997; Netzeband et al., 1999</td>
</tr>
<tr>
<td>NO</td>
<td></td>
<td>+</td>
<td>Fimiani et al., 1999; Mombouli et al., 1999</td>
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<tr>
<td>CB₂</td>
<td>G₁o</td>
<td>+</td>
<td>Bayewitch et al., 1995; Kobayashi et al., 2001</td>
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<td>−</td>
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<td>[Ca²⁺]ᵢ</td>
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<td>Zoratti et al., 2003</td>
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<tr>
<td>PLC/InsP₃</td>
<td></td>
<td>+</td>
<td>Zoratti et al., 2003</td>
</tr>
</tbody>
</table>

+ suggests activation, − suggests inhibition.
receptor within the pore formed within the transmembrane subfamily of GPCRs. Cannabinoid agonists interact with the G proteins. The proximal CB1 receptor intracellular C-terminal juxtamembrane C-terminal region contribute to the activation of loops and a putative fourth loop formed by palmitoylation at the T-type Ca2+ channel Anandamide

**Table 3**

<table>
<thead>
<tr>
<th>Signalling target</th>
<th>Cannabinoid</th>
<th>Effect</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>TRPV1 receptor</td>
<td>Anandamide</td>
<td>+</td>
<td>Smart et al., 2000; De Petrocellis et al., 2001</td>
</tr>
<tr>
<td>5-HT3 receptor</td>
<td>Anandamide</td>
<td>–</td>
<td>Barann et al., 2002; Oz et al., 2002</td>
</tr>
<tr>
<td></td>
<td>CP 55,940</td>
<td>–</td>
<td>Fan, 1995; Godlewski et al., 2003</td>
</tr>
<tr>
<td></td>
<td>WIN 55,212-2</td>
<td>–</td>
<td>Barann et al., 2002; Godlewski et al., 2003</td>
</tr>
<tr>
<td>nACh receptor</td>
<td>Anandamide</td>
<td>–</td>
<td>Oz et al., 2003</td>
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<tr>
<td></td>
<td>CP 55,940</td>
<td>–</td>
<td>Oz et al., 2004</td>
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<tr>
<td>NMDA receptor</td>
<td>Anandamide</td>
<td>+</td>
<td>Hampson et al., 1998</td>
</tr>
<tr>
<td>T-type Ca2+ channel</td>
<td>Anandamide</td>
<td>–</td>
<td>Chemin et al., 2001</td>
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<tr>
<td>TASK-1 channel</td>
<td>Anandamide</td>
<td>–</td>
<td>Maingret et al., 2001</td>
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<tr>
<td></td>
<td>CP 55,940</td>
<td>–</td>
<td>Maingret et al., 2001</td>
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<td>Na+ channel</td>
<td>Anandamide</td>
<td>–</td>
<td>Nicholson et al., 2003</td>
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<tr>
<td></td>
<td>WIN 55,212-2</td>
<td>–</td>
<td>Nicholson et al., 2003</td>
</tr>
</tbody>
</table>

+ suggests activation, – suggests inhibition.

cannabinoid targets is likely to expand. It remains however to be seen which of these are pure cannabinoid receptors, with endocannabinoids as their principal activators, or other elements which can be modulated by cannabinoids. Signal transduction mechanisms mediated by CB receptors are presented in Table 2 and discussed in detail below, followed by non-CB receptor-mediated mechanisms, also summarised in a table (Table 3).

**Signal transduction mechanisms of CB1 receptors**

**G protein coupling**

The CB1 cannabinoid receptor is a member of the rhodopsin subfamily of GPCRs. Cannabinoid agonists interact with the receptor within the pore formed within the transmembrane helical cluster (Mukhopadhyay et al., 2002). The three cytosolic loops and a putative fourth loop formed by palmitoylation at the juxtamembrane C-terminal region contribute to the activation of G proteins. The proximal CB1 receptor intracellular C-terminal domain (amino acids 401–417) is critical for G protein coupling and the distal C-terminal tail domain (amino acids 418–472) modulates the magnitude and kinetics of signal transduction (Nie and Lewis, 2001). Cannabinoid receptor stimulation of [35S]GTPγS binding has been quantified in rat membranes in the presence of excess GDP (Selley et al., 1996). In addition, most cannabinoid effects are sensitive to PTX implicating a CB1 receptor coupling to a G_{i/o} protein.

Evidence exists to suggest that CB1 receptors can interact with G_i under conditions of PTX treatment that prevents the receptor’s interaction with G_{i/o} proteins. Stimulation of cAMP was observed in rat cultured striatal neurons and in CB1-transfected CHO cells in response to the CB1 agonist HU-210 (Glass and Felder, 1997; Felder et al., 1998). This effect was observed separately. Interestingly, when these agonists were added together they augmented cAMP accumulation (Glass and Felder, 1997). Furthermore, Maneuf and Brotchie (1997) showed that high concentrations of WIN 55,212-2 could stimulate basal cAMP accumulation in a slice preparation of rat globus pallidus in the absence of forskolin and PTX. This effect was inhibited by SR 141716A.

Jarrahian et al. (2004) demonstrated that the expression of D2 receptors was sufficient to unmask a coupling of G_i to the CB1 receptor in human embryonic kidney (HEK) 293 cells transfected with both receptors. CP 55,940 inhibited forskolin-induced cAMP production in CB1-transfected cells in a PTX-sensitive manner, but stimulated cAMP production following D2 receptor expression. PTX had no effect on CB1-mediated cAMP accumulation. Interestingly, D2 receptor-induced inhibition of cAMP production was not compromised by CB1 receptor expression (Jarrahian et al., 2004). The mechanism for this response could be explained by an ability of D2 receptors to sequester G_{i/o} proteins—or subunits thereof—such that they will no longer be available to couple to CB1 receptors, thus leaving only coupling to G_i. Indeed, CP 55,940 inhibited cAMP production when cells were over expressed with G_{i}. In addition, a CP 55,940-mediated inhibition of cAMP was maintained following chronic exposure of cells to the D2 receptor agonist quinpirole (Jarrahian et al., 2004). It was also shown that a 10-fold increase in the concentration of CP 55,940 was required to stimulate cAMP production than inhibit its accumulation suggesting that the concentration of cannabinoids used may also determine the activation of either G_i or G_s (Jarrahian et al., 2004).

A CB1 receptor interaction with G_s has also been demonstrated in CHO cells expressing human CB1 receptors (Bonhaus et al., 1998). Pretreatment with PTX was used to observe receptor coupling with G_s. It was found that cannabinoid agonists (HU-210, CP 55,940, Δ9-THC, anandamide; order of potency for G_{i/o}-coupled effects) were markedly less efficacious when stimulating forskolin-stimulated cAMP production (G_i) than in inhibiting its formation (G_{i/o}). The CB1 antagonist SR 141716A equally inhibited both cannabinoid accumulation and inhibition of cAMP (Bonhaus et al., 1998). Thus, these findings indicate that there is specificity among CB1 receptor agonists in their relative abilities to activate G_i- and G_{i/o}-coupled transduction pathways.

Collectively the data strongly suggests that CB1 receptors may be dually coupled to both G_i and G_{i/o} proteins in some systems but the physiological significance of this needs further investigation.

**Regulation of adenylyl cyclase**

The first characterised CB1 receptor signal transduction response was the inhibition of adenylyl cyclase by micromolar concentrations of Δ9-THC in N18TG2 neuroblastoma cells (Howlett and Fleming, 1984). This response was blocked by PTX suggesting the involvement of G_{i/o} proteins (Howlett et al., 1986). Since then the functional inhibition of adenylyl
cAMP accumulation in CHO cells expressing exogenous CB₁ receptors (Patel et al., 2001; Rhee et al., 1998; Manef and Broachie, 1997). Busch et al. (2004) recently showed that anandamide evoked a concentration-dependent increase in cAMP in rat parotid glands. The CB₁ antagonist AM 281 inhibited this effect.

Nine distinct isozymes of adenylyl cyclase have been identified, which can be categorised into six distinct classes based on sequence and functional similarities (Patel et al., 2001): (a) AC type I (AC-I) is found mainly in brain, is stimulated by Ca²⁺/calmodulin and is inhibited by G₁₅α subunits and by G₁₅α; (b) AC-VIII is found only in brain and is only stimulated by Ca²⁺/calmodulin; (c) AC-II, AC-IV and AC-VII are activated by G₁₅α, providing that G₁₅α is present; (d) AC-V and AC-VI are highly expressed in brain and heart, and they are inhibited by G₁₅α and low levels of Ca²⁺; (e) AC-III is stimulated by a high concentration of Ca²⁺/calmodulin in the presence of G₁₅α; (f) AC-IX is expressed at high levels in skeletal muscle and brain and, as yet, is found to be affected by G₁₅α only.

The influence of the adenylyl cyclase isoform on the outcome of the response to cannabinoid agonists has been investigated (Rhee et al., 1998). Monkey kidney COS-7 cells, expressing exogenous CB₁ receptors, were transfected with each adenylyl cyclase isoform in turn and stimulated with the cannabinoid agonists HU-210 and WIN 55,212-2. AC-I, V, VI and VIII were shown to be inhibited by, whereas types II, IV and VII were stimulated by, CB₁ receptor activation. The inhibition of AC-III by the cannabinoids was only observed when forskolin was used as a stimulant while AC-IX was inhibited only marginally. These results suggest that, in addition to the dual coupling of cannabinoid receptors to Gₛ and G₁₅α, the contrasting effects of cannabinoids on adenylyl cyclase activity could also be attributed to the specific isoform present in different cellular preparations. For example the globus pallidus contains mRNA encoding for AC-II (Furuyama et al., 1993; Childers and Deadwyler, 1996). Studies in primary rat astrocyte cultures showed that Δ⁹-THC and HU-210 increased glucose metabolism, phospholipid metabolism and glycogen synthesis through the activation of MAP kinase (Sanchez et al., 1998b). The responses were PTX- and SR 141716A-sensitive. Two signal transduction pathways were proposed. The first involved the activation of PI3K, which in turn mediated tyrosine phosphorylation and activation of Raf. PI3K may signal via PKB as a mediator of MAP kinase (Sanchez et al., 1998b).}

**Regulation of mitogen-activated protein (MAP) kinase**

The MAP kinase pathway is a key signalling mechanism that regulates many cellular functions such as cell growth, transformation and apoptosis. Its activation is normally associated with the initial activation of a tyrosine kinase-linked receptor. This activates the intracellular G protein Ras and sets up a signalling cascade beginning with the activation of the serine/threonine kinase Raf (MAP kinase kinase kinase). Raf activates MAP kinase kinase (MEK) leading to the phosphorylation and activation of MAP kinase, which can phosphorylate various cytoplasmic and nuclear proteins.

CB₁ receptors have been shown to link positively to MAP kinase. In cultured U373MG human astrocytoma and CHO cells, expressing CB₁ receptors, HU-210 and CP 55,940 activated a p42/p44 MAP kinase (Bouaboula et al., 1995b; Galve-Roperh et al., 2002). These effects were PTX- and SR 141716A-sensitive (Bouaboula et al., 1995b). Furthermore, the activation of MAP kinase in CHO cells was linked to the activation of a Na⁺/H⁺ exchanger (NHE-1), a transporter involved in multiple cellular functions such as intracellular pH regulation and control of cell volume (Bouaboula et al., 1999). In vivo, acute administration of Δ⁹-THC induces a progressive and transient activation of p42/p44 MAP kinase in rat dorsal striatum and nucleus accumbens (Valijent et al., 2001), as well as in murine hippocampus (Derkinderen et al., 2003), striatum and cerebellum (Rubino et al., 2004). These effects were blocked by SR 141716A suggesting an involvement of the CB₁ receptor. CP 55,940, WIN 55,212-2, and anandamide and 2-AG have also been shown to stimulate p38 MAP kinase in rat and murine hippocampus. These effects were also mediated by the CB₁ receptor as they were blocked by SR 141716A and exhibited no stimulatory action in CB₁⁻/⁻ mice (Derkinderen et al., 2001).

Mechanisms for the induction of MAP kinase by CB₁ receptors have not been fully elucidated. Given the structure of cannabinoid receptors, and the sensitivity of responses to PTX, they are not believed to act as tyrosine kinase-coupled receptors (trk). Studies in primary rat astrocyte cultures showed that Δ⁹-THC and HU-210 increased glucose metabolism, phospholipid metabolism and glycogen synthesis through the activation of MAP kinase (Sanchez et al., 1998b). The responses were PTX- and SR 141716A-sensitive. Two signal transduction pathways were proposed. The first involved the activation of PI3K, which in turn mediated tyrosine phosphorylation and activation of Raf. PI3K may signal via PKB as a mediator of MAP kinase (Sanchez et al., 1998b). The second pathway was initiated by sphingomyelin hydrolysis, release of
the lipid second messenger ceramide and the subsequent activation of the Raf MAP kinase cascade (Sanchez et al., 1998b). A CB1 receptor-mediated production of ceramide by Δ9-THC has also been demonstrated in rat C6 glioma cells (Sanchez et al., 1998a; Galve-Roperh et al., 2000). Furthermore, the induction of a PI3K/PKB pathway, in response to cannabinoid receptor stimulation, was demonstrated in human prostate epithelial PC-3 cells (Sanchez et al., 2003). This in turn evoked the phosphorylation of p42/p44 MAP kinase. Interestingly, antagonists at both the CB1 and CB2 receptor inhibited the cannabinoid-mediated stimulation of PKB.

CB1 receptor stimulation may also regulate MAP kinase activity indirectly through its effects on cAMP accumulation. In MCF-7 cancer cells anandamide induced the activation of MAP kinase in a CB1-dependent manner (Melck et al., 1999). Forskolin and the cAMP analogue 8-Bromo-cAMP inhibited basal MAP kinase activity and significantly reduced the stimulatory effect of anandamide on MAP kinase activity compared to the endocannabinoid alone. In addition, anandamide affected cAMP levels at doses slightly lower than those required to stimulate MAP kinase (Melck et al., 1999). In rat hippocampal slices pretreatment with 8-Bromo-cAMP completely prevented the activation of MAP kinase by anandamide and 2-AG (Derkinderen et al., 2003). Finally, a study in N1E-115 neuroblastoma cells found that p42/p44 MAP kinase activation by WIN 55,212-2 was inhibited by forskolin while the PKA inhibitor H-89 enhanced MAP kinase phosphorylation (Davis et al., 2003). Collectively the data suggests that a decrease in cAMP levels, and consequently in PKA activity, may participate in the stimulatory effects of CB1 activation on the MAP kinase pathway.

MAP kinase activation can be linked to expression of immediate early genes, as has been demonstrated by a CB1-mediated expression of krox-24 in human astrocytoma cells (Bouaboula et al., 1995a). Intracerebroventricular injection of anandamide evoked an increase in c-Fos protein in rat brain with a generally similar distribution to that of functioning CB1 receptors (Patel et al., 1998). In MCF-7 cancer cells the anandamide-induced stimulation of MAP kinase was shown to exert a subsequent down-regulation on prolactin receptors and trk nerve growth factor receptors (Melck et al., 1999). This regulation of gene expression was thought to underlie the antiproliferative effects of anandamide in these cells. In mouse hippocampus Δ9-THC induced the expression of immediate-early gene products including krox-24, BDNF and c-Fos protein, which was prevented by the inhibition of MAP kinase (Derkinderen et al., 2003). BDNF and krox-24, in particular, are known to be important for synaptic plasticity (Derkinderen et al., 2003) suggesting that gene regulation, through the activation of MAP kinase, is an important physiological mechanism by which cannabinoids can modulate synaptic plasticity. Modulation of ion channels

The modulation of voltage-dependent ion channels (primarily N- and P/Q-type Ca2+ channels and KIR and A-type K+ channels) is thought to underlie the cannabinoind-induced inhibition of neurotransmitter release at presynaptic sites. The majority of these effects are mediated through the CB1 receptor, although there is evidence to suggest that cannabinoids modulate ion channel function directly. Evidence for both mechanisms (CB1-dependent and independent) are discussed in this section.

Anandamide, WIN 55,212-2 and CP 55,940 act via CB1 receptors to inhibit N-type VOCCs leading to a decrease in Ca2+ influx in NG108-15 cells (Mackie and Hille, 1992; Felder et al., 1993; Mackie et al., 1993). The response was blocked by prior treatment of the cells with PTX, demonstrating its mediation by Gi/o proteins, and was independent of the cAMP pathway, as the response was not reversed by the addition of 8-Bromo-cAMP (Mackie et al., 1993). Sugiuara et al. (1997) utilised fura-2 imaging to examine depolarisation-induced Ca2+ influx in high K+ depolarised NG108-15 cells. 2-AG and anandamide both attenuated the response. The N-type Ca2+ channel was presumed to account for the depolarisation-evoked increases in Ca2+ current because the N-type channel antagonist ω-conotoxin could block these responses (Caulfield and Brown, 1992; Mackie et al., 1993). In rat superior cervical ganglion neurons transfected with CB1 receptors, WIN 55,212-2 and CP 55,940 inhibited Ca2+ currents (Pan et al., 1996). These effects were both PTX- and ω-conotoxin-sensitive suggesting an inhibitory effect on N-type Ca2+ channels. In rat striatal neurons WIN 55,212-2 inhibited corticostriatal glutamatergic synaptic transmission in an SR 141716A- and PTX-sensitive manner (Huang et al., 2001). The inhibition of N-type Ca2+ channels was thought to mediate this effect as ω-conotoxin abolished the WIN 55,212-2-mediated synaptic inhibition.

Anandamide inhibited Q-type Ca2+ currents in AtT-20 pituitary tumour cells expressing exogenous CB1 receptors, which was inhibited by PTX (Mackie et al., 1995). Fura-2 studies in rat cortical and cerebellar brain slices showed that anandamide inhibited P/Q-type Ca2+ fluxes (Hampson et al., 1998). This response was SR 141716A- and PTX-sensitive, confirming its mediation by Gi/o protein-coupled CB1 receptors.

In cultured rat hippocampal neurons WIN 55,212-2, anandamide and CP 55,940 inhibited N- and P/Q-type Ca2+ currents in an SR 141716A- and PTX-sensitive manner (Twitchell et al., 1997; Shen and Thayer, 1998). Activation of CB1 receptors by WIN 55,212-2 (nanomolar concentrations) inhibited only a fraction (17%) of the whole-cell Ca2+ current, even though more than half of this current is carried by N- and P/Q-type Ca2+ channels (Shen and Thayer, 1998). Interestingly, the same study revealed that further inhibition of Ca2+ currents could be obtained using micromolar concentrations of WIN 55,212-2. In addition, the inactive stereoisomer WIN 55,212-3 (micromolar concentrations) also inhibited Ca2+ currents in an SR 141716A-insensitive manner. This clearly indicates that at micromolar concentrations the effects of WIN 55,212-2 are not mediated by CB1 receptors, which may suggest a direct effect of cannabinoids on Ca2+ channels. The inhibitory effect of cannabinoids on N-type Ca2+ channels in the hippocampus is in accordance with the observations of Wilson et al. (2001).
These authors demonstrated that WIN 55,212-2 was unable to mimic depolarisation-induced suppression of inhibition in rat hippocampal slices pretreated with α-conotoxin. This suggests that an endocannabinoid-mediated inhibition of N-type Ca\(^{2+}\) channels is required for a presynaptic reduction of GABA release.

L-type Ca\(^{2+}\) channels can also be regulated via CB\(_1\) receptor stimulation. WIN 55,212-2 inhibited L-type Ca\(^{2+}\) currents in cat cerebral arterial smooth muscle cells, in a PTX- and SR 141716A-sensitive manner (Gebremedhin et al., 1999). In retinal slices from larval tiger salamander activation of CB\(_1\) receptors by WIN 55,212-2 led to the inhibition of L-type Ca\(^{2+}\) channels in bipolar cells (Straiker et al., 1999).

T-type Ca\(^{2+}\) channels transfected in HEK 293 and CHO cells and endogenously expressed in NG108-15 cells were inhibited by anandamide (Chemin et al., 2001). This inhibitory effect was not mimicked by synthetic cannabinoids including WIN 55,212-2, CP 55,940 and HU-210 and was not blocked by SR 141716A. PL (PLA\(_2\), PLC and PLD) and PK (A and C) pathways were also not involved (Chemin et al., 2001). From these observations it was suggested that anandamide directly inhibits T-type Ca\(^{2+}\) channels.

Exogenously expressed CB\(_1\) receptors couple positively to endogenous GIRK channels in AtT-20 pituitary tumour cells (Mackie et al., 1995). WIN 55,212-2 activated an inward current, which showed inward rectification and was sensitive to low concentrations of Ba\(^{2+}\) (Mackie et al., 1995). In Xenopus oocytes, expressing CB\(_1\) receptors and either GIRK1 or GIRK4 channels, WIN 55,212-2 was able to enhance currents carried by these channels, in a Ba\(^{2+}\)-sensitive manner (McAllister et al., 1999). WIN 55,212-2 and CP 55,940 inhibited glutamatergic signalling in the mouse nucleus accumbens in an SR 141716A-sensitive manner (Robbie et al., 2001). Ba\(^{2+}\) blocked this inhibition suggesting a cannabinoid-mediated activation of GIRK channels as a mechanism for the inhibition of neurotransmitter release. Forskolin did not alter presynaptic CB\(_1\) actions suggesting that cannabinoids inhibited glutamate release independently from the AMP/PKA pathway (Robbie et al., 2001). Endogenously expressed inwardly rectifying K\(^{+}\) channels in HEK 293 cells transfected with CB\(_1\) were activated by WIN 55,212-2 and anandamide (Vasquez et al., 2003). These effects were inhibited by AM251 implicating a CB\(_1\)-dependent mechanism.

Importantly, Garcia et al. (1998) demonstrated that the WIN 55,212-2 evoked activation of GIRK channels in CB\(_1\)-transfected AtT-20 cells could be inhibited by stimulation of PKC. The finding that a mutation in the CB\(_1\) receptor prevented the ability of PKC to disrupt ion channel activation suggested that phosphorylation of the G-protein-coupled receptor could inhibit its activity (Garcia et al., 1998). Hence, the stimulation of PKC may provide a mechanism to restore neuronal excitability and synaptic strength when endocannabinoid levels are high.

In cultured hippocampal neurons WIN 55,212-2 increased voltage-dependent A-type outward K\(^{+}\) currents (I\(_{A}\)) (Deadwyler et al., 1995) and decreased voltage-independent D-type outward K\(^{+}\) currents (I\(_{D}\) or delay current) (Mu et al., 1999) in a concentration-dependent, SR 141716A- and PTX-sensitive manner. A cannabinoid-mediated reduction in cAMP/PKA was the mechanism shown to activate I\(_{A}\) (Hampson et al., 1995) and inhibit I\(_{D}\) (Mu et al., 1999). It was proposed that phosphorylation of the K\(^{+}\) channel inactivated I\(_{A}\) and therefore a decrease in PKA would act to reverse this process. As such, PKA inhibitors including IP-20, H7 and H8 mimicked the effects of WIN 55,212-2 on I\(_{A}\) (Hampson et al., 1995; Mu et al., 2000) while activators of PKA including 8-Bromo-cAMP and forskolin produced opposite effects to WIN 55,212-2 (Hampson et al., 1995). In addition, the phosphatase inhibitor okadaic acid blocked the stimulatory effects of WIN 55,212-2 on I\(_{A}\) (Mu et al., 2000). IP-20 inhibited I\(_{D}\) whereas 8-Bromo-cAMP potentiated I\(_{D}\) (Mu et al., 1999). This suggests that PKA-mediated phosphorylation activates D-type channels, which may be the reason why WIN 55,212-2 inhibits their activation.

Interestingly, WIN 55,212-2 acting through postsynaptic CB\(_1\) receptors was shown to decrease M-type K\(^{+}\) (I\(_{M}\)) currents in hippocampal CA1 neurons (Schweitzer, 2000). The author suggested that a CB\(_1\)-mediated increase in intracellular Ca\(^{2+}\) could be one of the mechanisms behind I\(_{M}\) inhibition. By reducing I\(_{M}\) cannabinoids diminish the ability of neurons to counteract depolarisations, favouring increased firing of action potentials, and thus induce hyperexcitability in the hippocampus (Schweitzer, 2000). Further studies are required to assess the physiological significance of this effect.

In cerebellar granule and COS-7 cells anandamide inhibited the acid-sensitive background K\(^{+}\) channel TASK-1 (Maingret et al., 2001). This effect was also observed with WIN 55,212-2 and CP 55,940 but not HU-210, 2-AG and Δ\(^{2}\)-THC and was not reversed with SR 141716A. This suggests a direct effect of anandamide on TASK-1 channels. TASK-1 is a member of a family of leak or background K\(^{+}\) channels that sets resting membrane potential (Maingret et al., 2001). Thus, inhibition of the channel would induce depolarisation and enhance excitability. Taking into account the localisation of TASK-1 in areas of motor control, such as motor neurons and cerebellar granular cells, Maingret et al. (2001) suggested that anandamide might influence motor behaviour through an interaction with TASK-1 but further studies are needed to support this hypothesis.

A study by Nicholson et al. (2003) demonstrated the ability of anandamide and WIN 55,212-2 to inhibit voltage-dependent Na\(^{+}\) channels (activated by veratridine) in mouse synaptosomes. The cannabinoids also blocked the veratridine-induced release of neurotransmitters from synaptosomes including GABA and glutamate. The CB\(_1\) antagonist AM 251 did not attenuate sodium channel inhibition. In addition, anandamide and WIN 55,212-2 were able to displace the binding of \(^{[3]}\)HJBTX-B to voltage-dependent Na\(^{+}\) channels (Nicholson et al., 2003). Together, the data suggests that cannabinoids can directly modulate the activity of voltage-dependent Na\(^{+}\) channels, depressing synaptic transmission in the brain and, in turn, reduce both excitatory and inhibitory transmitter release.

**Modulation of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\))**

\[ [\text{Ca}^{2+}]_{i} \] was increased by 2-AG and WIN 55,212-2 in NG108-15 cells (Sugiura et al., 1996a). This effect was both
PTX- and SR 141716A-sensitive. The PLC inhibitor U73122 was able to block the response suggesting an InsP_3-mediated release of Ca^{2+} from internal stores (Sumiki et al., 1997). The metabolically stable analogue of anandamide, methanandamide, was shown to deplete InsP_3-sensitive Ca^{2+} stores in primary cultures of striatal astrocytes, in a PTX-sensitive manner (Venancio et al., 1997). In cultured cerebellar granule cells methanandamide, WIN 55,212-2 and HU-210 augmented the Ca^{2+} signal in response to depolarisation induced by high K^+ or NMDA receptor stimulation (Netzeband et al., 1999). This response was mediated by postsynaptic CB_1 receptors as the effect was antagonised by SR 141716A and PTX. U73122 also blocked the augmented Ca^{2+} release suggesting a CB_1-mediated release of Ca^{2+} from InsP_3-sensitive stores (Netzeband et al., 1999). Collectively the data suggests that CB_1 receptor stimulation is coupled to PLC activation, through Gi/o proteins, in turn increasing levels of InsP_3 for the induction of Ca^{2+} release from internal stores.

In cultured human arterial endothelial cells anandamide evoked an increase in [Ca^{2+}]_i in an SR 141716A-sensitive manner (Fimiani et al., 1999). This increase in Ca^{2+} was coupled to the production of NO. Anandamide also induced a rise in [Ca^{2+}]_i in human umbilical endothelial cells (Mombouli et al., 1999). This increase was only marginally blocked by SR 141716A, insensitive to PTX and blocked by caffeine suggesting a release of Ca^{2+} from caffeine-sensitive intracellular stores. Anandamide also significantly increased NO synthase activity as determined by enhanced conversion of L-[{^3}H]arginine to L-[{^3}H]citrulline (Mombouli et al., 1999). These results suggest that CB_1-dependent and -independent increases in [Ca^{2+}]_i, and subsequent NO production may account for some of the vasodilator actions of anandamide. More generally, the increases in intracellular [Ca^{2+}]_i seen upon CB_1 receptor stimulation in a range of cells, including neurons (Sugiura et al., 1996a, 1997), seem at odds particularly with cannabinoid inhibition of neuronal excitability which is thought to be caused by VDCC inhibition, directly as well as indirectly. In addition to the NO synthase activation mechanism mentioned above, it is possible that subcellular localisation of signalling pathways could explain this paradox. For example, it can be envisaged that Ca^{2+} release from intracellular stores in excitable cells can be inhibitory through activation of Ca^{2+}-dependent K channels, leading to hyperpolarisation. This then would prevent the activation of VDCC and large Ca^{2+} influx.

In Madin–Darby canine kidney cells CP 55,940 increased [Ca^{2+}]_i in a concentration-dependent manner (Chou et al., 2001). CP 55,940 was shown to release Ca^{2+} from thapsigargin (an endoplasmic reticulum Ca^{2+} ATPase inhibitor)-sensitive stores in an InsP_3-independent manner, as the response was not altered by U73122. Filipeau et al. (1997) described an increase in [Ca^{2+}]_i in a hamster vas deferens smooth muscle cell line, DDT_1 MF-2 cells, following CB_1 receptor stimulation by Δ^9-THC. A cannabinoid-mediated release of Ca^{2+} from thapsigargin-sensitive stores was also established (Begg et al., 2001). Recently, this pathway has been further explored and was shown to involve Ca^{2+} influx mediated by arachidonic acid operated channels (Demuth et al., 2004).

**CB_2 signal transduction pathways**

Similar to CB_1, CB_2 receptors can modulate adenyl cyclase and MAP kinase activity, through their ability to couple to G_{i/o} proteins (Felber et al., 1995; Kobayashi et al., 2001). However, in contrast to CB_1, CB_2 receptor stimulation is believed not to modulate ion channel function, as seen in AtT-20 cells transfected with CB_2 receptors (Felber et al., 1995) and Xenopus oocytes transfected with CB_2 and GIRK1/4 (McAllister et al., 1999).

**Regulation of adenyl cyclase**

Cannabinoids were shown to inhibit adenyl cyclase activity in a concentration-dependent manner in CHO cells transfected with the CB_2 receptor (Bayewitch et al., 1995; Slipetz et al., 1995). This effect was PTX-sensitive suggesting signalling through G_{i/o} proteins. The same effect of cannabinoids was also observed in COS cells expressing CB_2 receptors (Slipetz et al., 1995). Again pretreatment with PTX blocked the cannabinoid-mediated inhibition. The inhibitory effect on cAMP production, induced by CB_2 stimulation, is thought to underlie, in part, the regulation of immune function by cannabinoids (Kaminski, 1996).

In contrast to CB_1 receptors CB_2 does not couple to G_{i/o}. Stimulation of cAMP accumulation by HU-210 and CP 55,940 was not observed after PTX treatment of CHO cells expressing the human CB_2 receptor (Glass and Felder, 1997; Calandra et al., 1999) suggesting that here is a clear difference between CB_1 and CB_2 receptor signalling.

**Regulation of MAP kinase**

Cannabinoids activate p42/p44 MAP kinase in CHO cells (Bouaboula et al., 1996) and HL-60 cells (Kobayashi et al., 2001) expressing the CB_2 receptor. In both studies the effects could be blocked with PTX and the CB_2 antagonist SR 144528. Cannabinoids were also shown to induce the expression of krox-24 through a PKC-dependent activation of MAP kinase (Bouaboula et al., 1996).

Treatment of human prostate epithelial PC-3 cells with either Δ^9-THC or methanandamide activated the PI3K/PKB pathway, which in turn induced translocation of Raf-1 to the membrane and phosphorylation of p42/p44 MAP kinase (Sanchez et al., 2003). SR 144528 was able to inhibit this induction suggesting the involvement of the CB_2 receptor.

Interestingly, Kaplan and Kaminski (2003) recently showed that WIN 55,212-2 concentration-dependently inhibited p42/p44 MAP kinase phosphorylation in stimulated mouse splenocytes. In addition, the MEK inhibitor PD098059 decreased evoked IL-2 production in the splenocytes (Kaplan and Kaminski, 2003). This suggests that a cannabinoid-mediated reduction of MAP kinase may inhibit IL-2 production in these cells and contributes a mechanism for immunosuppression by cannabinoids. Noteworthy is that these authors did not determine the cannabinoid receptor subtype involved in mediating this response (Kaplan and Kaminski, 2003).
although it is likely to be CB2-mediated as this is the most abundantly expressed cannabinoid receptor subtype in the immune system (Parolaro et al., 2002).

**Modulation of intracellular [Ca\(^{2+}\)]**

Anandamide initiated a rise in [Ca\(^{2+}\)]\(_i\) in calf pulmonary endothelial cells (Zoratti et al., 2003), which was sensitive to inhibition by the CB2 antagonist SR 144528 but not the CB1 antagonist SR 141716A. This increase resulted from the activation of PLC and a subsequent release of Ca\(^{2+}\) from InsP\(_3\)-sensitive stores (Zoratti et al., 2003).

**Interactions of cannabinoids with other receptor systems**

A number of investigations have demonstrated the ability of cannabinoids to modulate the activity of other receptor types. Therefore some of the behavioural effects of cannabinoids may occur through a synergistic action with other receptors and their signal transduction pathways. This section briefly describes some of these interactions.

**Opioid receptors**

Opioid compounds induce their pharmacological effects by activating \(\mu\)-, \(\delta\)- and \(\kappa\)-opioid receptors (Thompson et al., 1993; Mansour et al., 1995) and share several actions with cannabinoids including hypothermia, hypotension, motor depression and antinociception (Bloom and Dewey, 1978). Pharmacological interactions between the cannabinoid and opioid systems have been suggested, mainly concerning antinociception.

Smith et al. (1998) found that subcutaneous administration of \(\Delta^9\)-THC enhanced the antinociceptive potency of the opioid agonist morphine in the mouse tail-flick test. This action was SR 141716A-sensitive suggesting it was mediated through the CB1 receptor. The \(\kappa\)-opioid receptor antagonist nor-binaltorphimine and the \(\delta\)-opioid receptor antagonist naltrindole were able to block the antinociceptive effect caused by the combination of \(\Delta^9\)-THC and morphine (Pugh et al., 1996). They suggested that the antinociceptive effects of morphine, which are predominantly mediated by \(\mu\)-receptors, might be enhanced by \(\Delta^9\)-THC through activation of \(\kappa\)- and \(\delta\)-receptors (Pugh et al., 1996). This fact could be potentially useful in the treatment of pain.

Although the biochemical mechanisms involved in these interactions remain unclear several hypotheses have been formulated. Cannabinoids and opioids might interact at the level of their signal-transduction mechanisms (Manzanares et al., 1999) since opioid and cannabinoid receptors are coupled to similar intracellular signalling systems, i.e. inhibition of adenylyl cyclase activity and Ca\(^{2+}\) influx through activation of G proteins (Childers et al., 1992; Reisine et al., 1996). Consistent with this hypothesis, CP 55,940 and morphine were found to share a common cAMP signalling pathway in rat splenocytes (Massi et al., 2003). In vitro or chronic in vivo exposure to opiates and/or cannabinoids caused a desensitisation and cross-desensitisation in the ability of the respective drugs to inhibit forskolin-induced cAMP production. No synergistic inhibitory action was observed when CP 55,940 and morphine were administered together.

Cannabinoids may have a direct effect on the synthesis and release of endogenous opioids such as enkephalins and dynorphins. 5-day treatment with \(\Delta^9\)-THC significantly increased pro-opiomelanocortin gene expression (38%) in the arcuate nucleus of the rat hypothalamus (Corchero et al., 1997b), and increased prodynorphin (39%) and proenkephalin (34%) gene expression in rat spinal cord (Corchero et al., 1997a). This would suggest a cannabinoid-mediated increase in opioid peptide synthesis. Using microdialysis Valverde et al. (2001) showed that acute administration of \(\Delta^9\)-THC increased the release of enkephalin-like material in the nucleus accumbens of awake and freely moving rats suggesting cannabinoids can increase opioid release.

**Vanilloid TRPV1 receptor**

The TRPV1 receptor is a protein known to be primarily activated by noxious stimuli including heat, hydrogen ions and capsaicin, the ingredient found in chilli peppers (see Szallasi and Blumberg, 1999 for review). The receptor can be found on sensory neurons, where TRPV1 channel opening causes Ca\(^{2+}\) influx and neurotransmitter release.

Studies have demonstrated that anandamide can activate the TRPV1 receptor, although it is thought to do so by binding to sites on the cytosolic side of the receptor (De Petrocellis et al., 2001). Anandamide activated cloned TRPV1 ion channels expressed in HEK 293 cells, which could be blocked by the TRPV1 antagonist capsazepine (Zygmun et al., 1999). Anandamide could also mimic the effects of capsaicin to evoke vascular relaxation in arteries of the guinea-pig, in a capsazepine-sensitive manner (Zygmun et al., 1999), indicating that anandamide could activate TRPV1 channels in physiological preparations. This response could not be attributed to cannabinoid receptors because neither WIN 55,212-2 nor HU-210 were able to evoke the response while SR 141716A failed to attenuate the vasodilatory actions of anandamide. Anandamide has also been shown to activate TRPV1 receptors in rat hippocampal slices (Al-Hayani et al., 2001). Where WIN 55,212-2 normally reduced paired-pulse depression of population spikes in the CA1 region of the rat hippocampus (an indication of GABA release in the hippocampus), anandamide caused an increase. This effect was mimicked by capsaicin and blocked by capsazepine but not SR 141716A (Al-Hayani et al., 2001).

Due to the dual effects of anandamide on inhibitory CB1 and excitatory TRPV1 receptors Nemeth et al. (2003) investigated the effect of different concentrations of anandamide on neuropeptide release from sensory neurons of the rat trachea, which express both CB1 and TRPV1 receptors. Low concentrations of anandamide (10 \(\mu\)M) inhibited peptide release in an SR 141716A- and PTX-sensitive manner. High concentrations of anandamide (50–100 \(\mu\)M) increased the release of peptides and this response was inhibited by capsazepine. Moreover,
anandamide (10 μM) evoked release of peptides in the presence of SR 141716A, in a capsaicin-sensitive manner. This suggests that low concentrations of anandamide can induce neuropeptide release from peripheral sensory nerve terminals by TRPV1 activation if the inhibitory CB1 receptors are blocked. However, since activation of these receptors was only observed using high concentrations of anandamide the authors concluded that these potentiating effects are not likely to be relevant under physiological conditions (Nemeth et al., 2003).

The relative efficacy of anandamide at TRPV1 has been the subject of some controversy. Studies have shown that anandamide acts as a specific agonist at vanilloid receptors present in mouse trigeminal sensory neurons (Roberts et al., 2002) and in cultured DRG cells (Hwang et al., 2000). In contrast, studies have demonstrated that anandamide acts as a full agonist in HEK 293 cells transfected with human TRPV1 (Smart et al., 2000) and rat recombinant TRPV1 (Ralevic et al., 2001), as well as in isolated rat mesenteric arteries expressing endogenous TRPV1 receptors (Ralevic et al., 2001). These latter experiments used indirect measures of channel activity, i.e. elevation of total [Ca\(^{2+}\)]. It was proposed that these increases in intracellular Ca\(^{2+}\) may be due to amplification of the initial influx of Ca\(^{2+}\) through TRPV1 receptors, via mechanisms involving release of Ca\(^{2+}\) from intracellular stores, activation of store-operated Ca\(^{2+}\) channels and Ca\(^{2+}\)-mediated stimulation of PLC (Roberts et al., 2002). Therefore the high potency of anandamide, as determined by increases in [Ca\(^{2+}\)], may not be a true reflection of the action of anandamide at TRPV1 receptors, i.e. Ca\(^{2+}\) entry through ion channels.

**Serotonin (5-HT\(_3\)) receptor**

The 5-HT\(_3\) receptor is a ligand-gated ion channel that is associated with mood, pain and emesis (Greenshaw, 1993). Early work by Fan (1995) in rat nodose ganglion neurons showed that anandamide, WIN 55,212-2 and CP 55,940 inhibited 5-HT-induced currents in a concentration-dependent manner. The inward current was sensitive to blockade by the specific 5-HT\(_3\) receptor antagonist MDL72222 suggesting a cannabinoid-mediated inhibition of 5-HT\(_3\) currents.

A more recent study looked at the effect of cannabinoids in HEK 293 cells transfected with the human 5-HT\(_3\)A receptor only (i.e. no CB receptor expression) (Barann et al., 2002). The 5-HT-induced currents were inhibited by \(\Delta^9\)-THC, WIN 55,212-2 and anandamide in a concentration-dependent manner. The WIN 55,212-2-induced inhibition was not altered by SR 141716A and \([\text{H}]\text{CP} 55,940\) was shown not to bind to HEK 293 cells further suggesting that the effects are not mediated through either CB\(_1\) or CB\(_2\) receptors. Additional binding studies showed that WIN 55,212-2, anandamide and SR 141716A did not affect binding of the 5-HT\(_3\) specific ligand \([\text{H}]\text{GR65630}\) to the 5-HT\(_3\) receptor but 5-HT caused a concentration-dependent inhibition. This suggests that the cannabinoids do not interact directly with the agonist binding site of the 5-HT\(_3\) receptor, but may instead act allosterically at a 5-HT\(_3\) modulatory site (Barann et al., 2002).

In vivo experiments were conducted to investigate whether cannabinoids also modulate the activity of rat peripheral 5-HT\(_3\) receptors on the terminals of cardiopulmonary afferent C-fibres (Godlewski et al., 2003). In the presence of SR 141716A injection of the 5-HT\(_3\) agonist phenylbiguanide or capsaicin caused an immediate decrease in heart rate and mean arterial blood pressure. CP 55,940 and WIN 55,212-2 attenuated the 5-HT\(_3\)-induced bradycardia but failed to affect the capsaicin-evoked bradycardia (Godlewski et al., 2003). These data support that of Barann et al. (2002) who suggested that cannabinoids might be mediating their effects through an allosteric interaction with the 5-HT\(_3\) receptor. This modulatory site may be a potential target for the development of new analgesic and antiemetic drugs in the future.

**N-methyl-D-aspartate (NMDA) receptor**

Areas rich in CB\(_1\) receptors including the basal ganglia and hippocampus also show a high expression of NMDA receptors (a glutamate-sensitive cationic channel involved in excitatory neurotransmission), which are important in the control of movement and memory formation (Ossowska, 1994; Thorat and Bhargava, 1994). Cannabinoids have been shown to have dual effects on NMDA receptor activity. Hampson et al. (1998) initially showed that \(\Delta^9\)-THC and anandamide inhibited NMDA-induced Ca\(^{2+}\) influx in rat cortical and cerebellar slices. This effect was CB\(_1\)-mediated as it could be blocked by SR 141716A and PTX and involved the inhibition of P/Q-type Ca\(^{2+}\) channels. Interestingly, when the CB\(_1\) receptor component was blocked, anandamide but not \(\Delta^9\)-THC produced a stimulatory effect on NMDA-induced Ca\(^{2+}\) responses including rat cortical, cerebellar and hippocampal slices. This effect was mimicked in *Xenopus* oocytes transfected with NMDA receptors, where both anandamide and the stable analogue methanandamide dose-dependently potentiated NMDA-induced currents (Hampson et al., 1998). This latter result suggests a direct effect of anandamide on NMDA receptors.

Methanandamide, WIN 55,212-2 and HU-210 have also been demonstrated to enhance NMDA-evoked Ca\(^{2+}\) flux in primary cerebellar cultures (Netzeband et al., 1999). This effect was antagonised by SR 141716A, PTX and U73122 suggesting a CB\(_1\)-mediated release of Ca\(^{2+}\) from InP\(_3\)-sensitive stores was involved. Importantly, blockade of the PLC pathway unmasked a CB\(_1\)-mediated inhibition of the NMDA-evoked Ca\(^{2+}\) response (Netzeband et al., 1999).

**nAChRs**

nAChRs are ligand-gated ion channels that play a critical role in fast synaptic transmission throughout both the peripheral and central nervous system (see Role and Berg, 1996 for review). Physiologically, these receptors mediate neuromuscular transmission and hence movement (muscle-type AChRs) as well as all autonomic activity (neuronal-type AChRs).
Evidence is emerging to suggest that cannabinoids may be able to modulate the activity of nAChRs in a cannabinoid receptor-independent manner. Anandamide and 2-AG were shown to inhibit nicotine-evoked currents in *Xenopus* oocytes transfected with α7 nAChRs (Oz et al., 2003, 2004). The response to anandamide was insensitive to SR 141716A, the CB2 antagonist SR 144528, PTX and 8-Bromo-cAMP. WIN 55,212-2 and Δ9-THC had no effect on nicotine-evoked currents while CP 55,940 only significantly inhibited currents at concentrations exceeding 3 μM (Oz et al., 2004). In the same study arachidonic acid dose-dependently inhibited currents evoked by α7 nAChRs, although the metabolically stable analogue methanandamide and 2-AG showed a higher potency for inhibiting currents (Oz et al., 2004). This suggests that it was the intact endocannabinoid and not the metabolite that altered receptor function.

nAChRs are known to exhibit a number of binding sites that recognise a variety of endogenous non-competitive inhibitors, including fatty acids (Arias, 1998). Hence, it is interesting to speculate that the cannabinoid receptor-independent effects of anandamide and 2-AG on nAChR function may result from a presence of inhibitory binding sites on the channel, which specifically recognise endocannabinoids.

### Concluding remarks

It can be seen from the brief literature overview presented here that there are at least four elements that complicate the picture with regard to cannabinoid signalling. Firstly, natural, synthetic, or endogenous cannabinoid ligands might act on cannabinoid receptors but these might not be their only—or even primary—target. An example of this is anandamide, which some authors would perhaps describe as primarily a vanilloid agonist. Secondly, there are likely to exist more types of cannabinoid receptors than the CB1 and CB2 receptors and some authors would perhaps describe as primarily a vanilloid agonist. Secondly, there are likely to exist more types of cannabinoid receptors but these might not be their only—or even primary—target. An example of this is anandamide, which among other things opens up the possibility of a great number of interactions with other signalling systems. Finally, the pharmacology of cannabinoid-mediated actions is far from straightforward, indicated by emerging reports of species and tissue differences in actions of ligands. Although it is a sign that the cannabinoid signalling field is healthy and expanding, it is clear that care must be taken in the future use of the pharmacological tools available. It appears that after some years where cannabinoid signalling seemed relatively straightforward, a new phase of research in this field, complex but exciting, lies ahead.

### References


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