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The Pharmacology of the Cannabinoid System—A Question of Efficacy and Selectivity

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Abstract Our knowledge of the function of the cannabinoid system in the body has been aided by the availability of pharmacological agents that affect its function. This has been achieved by the design of agents that either directly interact with the receptor (agonists and antagonist/inverse agonists) and agents that indirectly modulate the receptor output by changing the levels of the endogenous cannabinoids (endocannabinoids). In this review, examples of the most commonly used receptor agonists, antagonists/inverse agonists, and indirectly acting agents (anandamide uptake inhibitors, fatty acid amide hydrolase inhibitors, monoacylglycerol lipase inhibitors) are given, with particular focus upon their selectivity and, in the case of the directly acting compounds, efficacy. Finally, the links between the endocannabinoid and cyclooxygenase pathways are explored, in particular, with respect to agents whose primary function is to inhibit cyclooxygenase activity, but which also interact with the endocannabinoid system.

Keywords Cannabinoid receptor · Anandamide · 2-Arachidonoylglycerol · Fatty acid amide hydrolase · Monoacylglycerol lipase · Non-steroidal anti-inflammatory drugs

Introduction

There is evidence in early texts that cannabis was used for medicinal purposes as long ago as the sixteenth century BC, and a stable constituent of cannabis was recovered from a 14-year old girl who died in childbirth in the fourth century AD [1]. The structure of the main psychoactive ingredient of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) was not identified until 1964 [2]. Since then, the pace of cannabinoid research has picked up considerably, and there is now an intensive effort worldwide both to understand how endogenous, plant-derived, and synthetic cannabinoid compounds affect the body, and whether such compounds have therapeutic usefulness.

Signaling molecules in the body require pathways and mechanisms for their synthesis, storage [unless they are made upon demand, which is the case for endogenous cannabinoid (endocannabinoid) molecules], release, and removal, as well as appropriate receptor molecules with which to transfer the information. The cannabinoid system is no exception, and most stages in these pathways have been, or are being, elucidated (for reviews, see [3, 4]). Provided that the mechanism in question exists (an obvious prerequisite but one that is the source of considerable controversy with respect to the release and reuptake of the endocannabinoids), it is in theory amenable to pharmacological manipulation, and there are now a variety of agents available that can affect cannabinoid signaling, either directly via actions upon cannabinoid (CB) receptors or indirectly via modulation of the two main endocannabinoids anandamide (AEA, arachidonoylethanolamide) and 2-arachidonoylglycerol (2-AG). The aim of the present review is to describe the pharmacology of the endocannabinoid system to set in context the other articles in this issue of *Molecular Neurobiology.* A particular focus has been made on the selectivity of the compounds involved, as this is a key to the interpretation of their effects. Authors interested in the therapeutic promise of such agents are referred to [4–6] for reviews.

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At the outset, a note should be made concerning the cannabinoid receptors. Currently, the International Union of Pharmacology (IUPHAR) database lists two, CB_1 and CB_2 (http://www.iuphar-db.org/index.html), and the present review has primarily considered actions upon these receptors. However, evidence is emerging to suggest that additional receptors, such as the endothelial and central nervous system (CNS) "non-CB_1, non-CB_2" receptors and the GPR55 orphan receptor, can be activated by plant-derived, synthetic, and/or endogenous cannabinoids [7, 8], as can receptors that are plainly not part of the cannabinoid receptor "family," such as 5-HT_3 receptors [9] and TRPV1 (vanilloid) receptors [10]. Hopefully, further research will indicate as to whether or not the "non-CB_1, non-CB_2" receptors and the GPR55 orphan receptor should be formally included in the CB receptor family.

Directly Acting Cannabinoid Ligands

Over the years, a variety of methodologies have been utilised to establish affinities, potencies, and efficacies of directly acting cannabinoid ligands. Early methods for detecting cannabinoid action, such as the dog ataxia test [11], have been replaced by a battery of techniques ranging from molecular modelling strategies to biochemical [e.g., radioligand binding, cyclic AMP (cAMP) inhibition, [^{35}S] GTPγS binding] and behavioural (e.g., the mouse tetrad model) tests ([12]; for recent examples, see [13, 14]).

In addition to the endogenous and plant-derived cannabinoid compounds of which AEA, 2-AG, and Δ^9-THC are the most intensively studied, there are now a very large number of synthetic compounds available that directly affect the activity of CB_1 and CB_2 receptors (for recent reviews, see [15–17]; see also [18] for examples of compounds that affect the activity of the CB_1 receptor via an allosteric interaction). Of these, the most commonly used synthetic compounds are shown in Fig. 1. I have used the rather old-fashioned terms "mimetic" and "lytic" deliberately, in part, to salute the use of these compounds in a number of key studies elucidating the function of CB receptors and, in part, to avoid difficulties with the classification of efficacy of compounds like rimonabant and AM1241 (see below). Without such compounds, our knowledge of the role of the cannabinoid system in processes as diverse as reproduction, regulation of appetite, conditioned drug seeking, inflammation and pain processing, and retrograde signaling in the brain would be limited (reviews, see [6, 19–25]).

Two important issues for any receptor-active compound are efficacy and selectivity. With respect to efficacy, the standard theory describing the interaction of ligands and receptors and the responses thereafter consider a two-state model, whereby the ligand shifts the receptor into an active

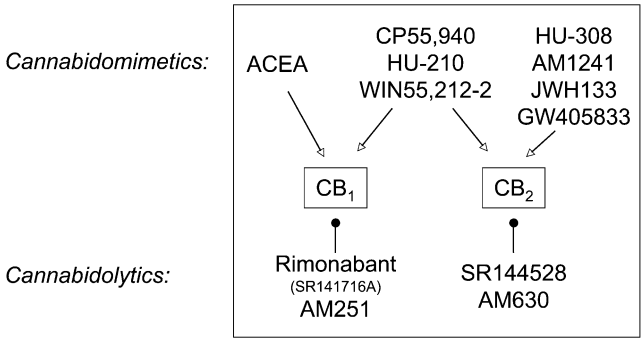

Fig. 1 Some of the most commonly used synthetic CB receptor ligands. *ACEA N*-(2-Chloroethyl)-5*Z*,8 *Z*,11 *Z*,14 *Z*-eicosatetraenamide, *AM251 N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1 *H*-pyrazole-3-carboxamide, *AM630* 6-iodo-2-methyl-1-[2-(4-morpholinyl) ethyl]-1H-indol- 3-yl](4-methoxyphenyl) methanone, *AM1241* 2-iodo-5-nitrophenyl)-(1-(1-methylpiperidin-2-ylmethyl)-1H-indol-3-yl)methanone, *GW405833* 1-(2,3-dichlorobenzoyl)-5-methoxy-2-methyl-(2-(morpholin-4-yl) ethyl)-1H-indole, *HU-210* (6a*R*)-trans-3-(1,1-dimethylheptyl)-6a, 7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol, *HU-308* 4-[4-(1,1-dimethylheptyl)-2,6-dimethoxyphenyl]-6, 6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol, *JWH133* (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[b, d]pyran, *rimonabant (SR141716A)N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide), *SR144528 N*-[(1S)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide, *WIN 55,212-2* (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate

or an inactive conformational state, as appropriate [26]. In this respect, there has been much debate as to whether rimonabant and other "cannabinolytic" compounds are inverse agonists or antagonists, and several neutral antagonist compounds have been claimed. In brief, there is good evidence that, in recombinant systems, rimonabant, AM251, SR144528, and AM630 produce inverse agonist effects, whereas in vivo, the distinction between the ability of a compound to silence a constitutively active receptor and to block an endocannabinoid tone is more difficult to demonstrate (reviews, see [5, 27]). However, the situation is more complicated than that, and there are a number of observations that do not fit into the standard two-state model:

- In Chinese hamster ovary cells transfected with the human CB_1 receptor (but not in the wild type cells), rimonabant not only acted as an inverse agonist to reduce the activity of mitogen-activated protein kinase, but also blocked the stimulation of this signaling pathway by insulin and insulin-like growth factor 1 [28]. The authors rationalized these and other data by suggesting a three-state model consisting of an active state of the receptor, an inactive state not coupled to the G protein, and an inactive state coupled (and thereby, rendering the latter unavailable for other receptor

systems) to the G protein ([28]; see also [29]). A similar property was also demonstrated for the CB_2 receptor [30].

- It is now well established that CB_1 receptors can couple to more than one G protein and to a variety of downstream signaling pathways [12]. In two elegant papers, Mukhopadhyay and Howlett [31, 32] demonstrated that different domains of the CB_1 receptor associate with different $G_{\alpha i}$ protein subunits and that the apparent efficacies of methanandamide and desacetyllevanantradol (but not WIN55,212-2 or rimonabant) were dependent upon the G_{α} subunit in question. Using Chinese hamster ovary cells transfected with the human CB_1 receptor, Bonhaus et al. [33] found that the relative efficacies, but not the rank order of potencies, of AEA, CP55,940, HU-210, Δ^9-THC, and WIN55212-2, were dependent upon the cellular response being measured. Thus, for example, the maximum inhibition of forskolin-stimulated cAMP production produced by CP55,940 was 92% of that seen with WIN55,212-2. In contrast, the ability of the CP55,940 to stimulate the response to forskolin in pertussis toxin-treated cells reached a maximum value of 45% of that for WIN55,212-2 [33]. More recently, Shoemaker et al. [34] utilized Chinese hamster ovary cells transfected with the human CB_2 receptor and found that the relative potencies of 2-AG, noladin ether, and CP55,940 varied depending upon the signal transduction pathway being measured. Yao et al. [35] have demonstrated in recombinant systems that AM1241 can act as a partial agonist or as a neutral antagonist depending upon the functional assay used. These observations have a theoretical basis in the "protean agonist" and "agonist-tracking" hypotheses, which postulate that G-protein-coupled receptors can, in fact, adopt multiple conformations and that the properties of the ligand determine the relative proportion of the conformations and, hence, the response pattern [36]. Evidence of such multiple conformations is beginning to appear in the literature [37, 38]. Whether or not such phenomena are involved in the disparity between the ability of a series of agonists to desensitise CB_1 receptors and their relative efficacies [39] awaits elucidation.

The above discussion clearly indicates that the simple two-state model is not sufficient to describe the interactions of ligands with CB receptors and that the observed efficacy of a compound is highly dependent upon the response measured, a factor of considerable importance for the design of predictive high-throughput screening strategies. The situation is further complicated by factors such as the ability of CB receptors to cross-talk with other pathways to produce a hypersensitization in addition to the heterologous desensitization described above [40] and/or to form homo- and heterodimers (review, see [41]). As well put by Rang et al. [26] in their discussion of receptor theory in general, "somehow the molecules always seem to remain one step ahead"!

A second, but separate, issue is the selectivity of the compounds. In theory, an effect of a compound like CP55,940 that is blocked by rimonabant could reasonably be ascribed to a CB_1-mediated event. Similarly, an action produced by WIN55212-2 but not its purportedly inactive enantiomer WIN55212-3 can be interpreted as a CB receptor-mediated event (see e.g. [42]), although there is evidence in transfected cells that WIN 55,212-3 is, in fact, a weak neutral antagonist (pA_2 value, 6.1) at human CB_2 receptors, a partial inverse agonist (pIC_{50} value, 5.5) at human CB_1 receptors, and can interact with the human MT_1 receptor melatonin at low micromolar concentrations [43]. Interpretation of the effects of CB receptor agonists assumes absolute selectivity of the compounds for the receptors in question. In many cases, the assumption is more than justified, particularly when pharmacological data is coupled with the use of animals with genetic deletions of the receptor in question. Two recent examples for the CB_1 receptor are the studies of Osei-Hyiaman et al. [44] and Kawamura et al. [45]. In the former, it was found that de novo liver fatty acid synthesis, both in vitro and in vivo, was increased by HU210 and decreased by rimonabant, and that the effect of HU210 was not seen in $CB_1^{-/-}$ mice [44]. In the latter, it was shown that WIN55212-2 suppressed excitatory synaptic transmission in hippocampal slices from 10- to 19-day-old C57BL/6 mice in a manner blocked by AM251. Once again, the effect was not seen in $CB_1^{-/-}$ mice [45].

Life would be rather dull if rules did not have exceptions. The exceptions in this case refer to the ability of directly acting cannabinoid receptor ligands to interact with other targets. As an example of this, Table 1 [9, 46–60] lists a series of observations made with WIN55212-2 and WIN55212-3 that are not consistent with actions, at the doses or concentrations shown, at CB_1 or CB_2 receptors. Although many of the effects were observed at high (micromolar) concentrations, WIN55212-2 is by no means the only compound to behave in this manner, and actions of Δ^9-THC, rimonabant, SR144528, and AEA upon a variety of other receptors and channels have been reported (see, e.g., [5, 7, 8] for recent reviews; see also the section below with respect to SR144528). The ability of AEA to interact with TRPV1 (vanilloid) receptors [10] has been of particular interest, and it has been suggested that AEA has a dual effect on capsaicin-sensitive primary sensory neurones, whereby a CB_1 receptor-mediated inhibitory effect in normal conditions is compromised by a TRPV1-induced excitatory effect in inflammatory conditions [61, 62].

Table 1 Effects of WIN55212-2 that cannot directly be attributed to actions at CB_1 or CB_2 receptors

Finding	Reference
WIN55212-2 and WIN55212-3 ↑ polarization of 1,6-diphenylhexatriene in rat brain synaptic plasma membranes at concentrations ≥5 μM, consistent with a change in the degree of membrane ordering	[46]
WIN55212-2 (IC_{50} value, 600 nM) more potent and efficacious than CP55,940 to ↓ isoprenaline-stimuated cAMP accumulation in cultured striatal astrocytes from embryonic mice. WIN55212-3 without effect. The action of WIN55212-2 not blocked by rimonabant. CB_2 receptor antagonist/inverse agonists not investigated	[47]
WIN55212-2 ↑ [^{35}S]GTPγS binding in membranes from $CB_1^{-/-}$ mouse brains (EC_{50} value, 1.8 μM). The effect of WIN55212-2 not sensitive to rimonabant unless high (μM) concentrations were used. Stimulatory effect of WIN55212-2 seen in membranes from brain stem, cortex, diencephalon, hippocampus, and midbrain, but not basal ganglia, cerebellum or spinal cord	[48]
WIN55212-2 (1 μM) ↓ evoked excitatory postsynaptic currents recorded in CA1 hippocampal pyramidal cells by ~50% in both $CB_1^{+/+}$ and $CB_1^{-/-}$ mice, effects reversed by rimonabant (1 μM). WIN55212-2 ↓ evoked inhibitory postsynaptic currents in the $CB_1^{+/+}$ but not the $CB_1^{-/-}$ mice. Further studies indicated that for the wild-type animals, the effect on evoked excitatory postsynaptic currents was dependent upon the strain of mouse used	[49, 50]
WIN55212-2 ↓ 5-HT-induced currents in excised outside–out patches of HEK293 cells expressing the human 5-HT_{3A} receptor (IC_{50} value, 104 nM); WIN55212-3 without effect. No effect of WIN55212-2 upon the binding of [^{3}H]GR65630 to the 5-HT_{3A} receptor, suggesting an allosteric modulation of receptor function	[9]
WIN55212-2, but not WIN55212-3 ↓ lipopolysaccharide-induced TNFα release from cultured neonatal rat cortical microglial cells. Concentrations of 1, 5, and 10 μM produced reductions of ~30, ~60, and ~90%, respectively. Effects of WIN55212-2 not blocked by either rimonabant, AM251, SR144528, or pertussis toxin treatment	[51]
WIN55212-2 and WIN55212-3 equipotent in stimulating glycerol release from male rat adipocytes. EC_{50} values 420 and 250 nM, respectively. Response to WIN55212-2 reduced by 10 μM but not 1 μM AM251; RT-PCR indicated no obvious expression of CB_1 or CB_2 receptors	[52]
WIN55212-2 ↓ [^{3}H]glutamate release from rat hippocampal synaptosomes, albeit with a high IC_{50} value (3.5 μM). Not inhibited by rimonabant (1 μM), AM251 (1μM), or capsazepine (10–30 μM). Twenty micromolar WIN55212-2 also ↓ release from hippocampal and striatal synaptosomes from $CB_1^{-/-}$ mice	[53, 54]
WIN55212-2 ↓ veratridine-induced depolarization of mouse brain synaptoneurosomes (IC_{50} values 21 μM), veratridine-induced γ-aminobutyric acid (GABA) and L-glutamate release from purified synaptosomes (IC_{50} values, 14 and 12 μM, respectively), and the binding of [^{3}H]batrachotoxin-B to synaptoneurosomal Na^+ channels (IC_{50} value, 20 μM). Effects not blocked by 2 μM AM251	[55]
WIN55212-2 (0.125 mg/kg i.v.) ↓ spiking probability on rat basolateral amygdala neurons in response to medial prefrontal cortex stimulation; effect blocked by rimonabant (1 mg/kg i.v.) but not by AM251 (1–2 mg/kg i.v.). Similar result found for the inhibitory effect of WIN55212-2 on the spontaneous discharge of these neurons	[56]
WIN55212-2 ↑ basal (EC_{50} value 26 μM) and ↓ K^+-evoked (IC_{50} value 1.7 μM) CGRP release from cultured rat trigeminal neurons. WIN55212-3 had similar effects, and the responses to WIN55212-2 were not blocked by either rimonabant or AM251	[57]
WIN55212-2 ↓ 5-HT and dopamine uptake into rat neocortical synaptosomes. The maximal inhibition of 5-HT was ~50% with a K_i value of 2 μM. Dopamine uptake was inhibited by ~70% with a K_i value of 440 nM. The inhibition of either uptake produced by 20 μM WIN55212-2 was not blocked by 20 μM AM251	[58]
WIN55212-2 (≥10 μM) but not WIN55212-3 ↓ IL-1 induction of adhesion molecules ICAM-1 and VCAM-1 in 1321N1 astrocytoma cells. Effect not blocked by rimonabant (10 μM), SR144528 (10 μM), or pertussis toxin treatment	[59]
WIN55212-2 (≥1 μM) ↓ neutrophil transmigration across TNFα-stimulated ECV304 cells. Effect mirrored by an ↓ IL-8 release from ECV304 cells and not prevented by AM251 or AM630	[60]

Some authors refer to WIN55212-2 and WIN55212-3 as the (R)-/(+)- and (S)-/(−)- enantiomers of WIN55212-2, respectively.
CGRP Calcitonin gene related peptide, *GR65630* (3-5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone, *ICAM-1* intracellular adhesion molecule-1, *TNF*α tumor necrosis factor α, *VCAM-1* vascular cell adhesion molecule-1

Indirectly Acting Compounds

Indirectly acting compounds exert their effects by changing the concentrations of the endocannabinoids. This can be achieved, at least in theory, by modulating the synthesis, release, and removal mechanisms for the endocannabinoids. At present, no selective inhibitors of AEA synthesis have been disclosed, and recent reports of multiple synthetic pathways for this endocannabinoid [63, 64] do not make the situation easier. However, tetrahydrolipstatin potently inhibits the synthesis of 2-AG and has been used as such to demonstrate the involvement of this endocannabinoid in retrograde signaling in the brain [65, 66]. Two compounds, O-3640 [octadec-9-enoic acid-1-(fluoro-methyl-phosphoryloxymethyl)-propylester] and O-3841 [octadec-9-enoic acid 1-methoxymethyl-2-(fluoro-methyl-phosphinoyloxy)-ethyl ester], have been reported to inhibit the activity of the 2-AG synthetic enzyme sn-1-selective diacylglycerol lipase α with submicromolar potencies and with good selectivity over monoacyl- and triacylglycyl lipases, CB_1, CB_2, and

AEA synthetic and degradative enzymes [67]. However, these compounds, unlike tetrahydrolipstatin, did not block ionomycin-induced 2-AG synthesis in intact cells [67], and so, are probably not useful for in vivo studies. Nevertheless, the identification of lead compounds preventing 2-AG synthesis is a useful start.

No compounds selectively affecting the release of endocannabinoids have, to my knowledge, been disclosed, although there is evidence that compounds blocking the reuptake of AEA can also affect the release process [68–70]. A major obstacle to development of compounds, however, is uncertainty as to the nature of the release process itself (see [71] and references therein).

The removal of AEA and 2-AG is achieved primarily by a process of cellular accumulation followed by hydrolysis to give arachidonic acid. Whereas the enzymes involved in these processes are largely identified, the mechanisms involved in the uptake of these endocannabinoids are a matter of considerable controversy at present, suggestions in the case of AEA ranging from a process of facilitated diffusion [72, 73] to rate-limited diffusion driven by its intracellular hydrolysis (see [74]; for recent reviews, see [75, 76]). Several compounds, almost all acyl derivatives, have been reported to inhibit AEA uptake and to have biological activity in vivo. These include AM404 (*N*-(4-hydroxyphenyl)arachidonylamide), VDM11 (*N*-(4-hydroxy-2-methylphenyl) arachidonoyl amide), UCM707 ((5Z,8Z,11Z,14Z)-*N*-(3-furanylmethyl)-5,8,11,14-eicosatetraenamide), and OMDM-2 ((9Z)-*N*-[1-((*R*)-4-hydroxbenzyl)-2-hydroxyethyl]-9-octadecen-amide) [77–80]. These compounds may block the uptake of AEA by more than one mechanism, although a key property seems to be their interaction with the hydrolytic enzyme fatty acid amide hydrolase (FAAH) [74]. As with the directly acting cannabinoid compounds discussed above, a key issue is the selectivity of these compounds. Indeed, the most widely used of the uptake inhibitors, AM404, has additional effects upon CB_1 receptor recognition sites, TRPV1 receptors, sodium channels, cyclooxygenase (COX) enzymes, calcium homeostasis, and cell viability at concentrations similar to those (low μM) that are required for inhibition of uptake [55, 81–86]. Indeed, arachidonoyl compounds can affect membrane ordering at these concentrations [46]. Such a variety of actions can contribute to the in vivo profile of AM404. Thus, for example, whereas the ability of AM404 to reduce the first phase of paw licking after local formalin treatment of mice was blocked by rimonabant but not by the TRPV1 antagonist capsazepine [87], the reverse was true for the ability of this compound to reduce the hyperkinesias produced by intrastriatal injections of 3-nitropriopionic acid in the rat [88]. The other three compounds are more selective (although they also affect cell viability at pharmacologically relevant concentrations [86], and VDM11 has potent effects upon calcium "spiking" in hippocampal neurons [83]) and also show biological activity in vivo (review see [89]). Ideally, more potent and, preferably, non-acyl compounds should be used, but such compounds are few and far between. Indeed, the only such compounds that has been reported in detail is LY2183240 (5-biphenyl-4-ylmethyl-tetrazole-1-carboxylic acid dimethylamide), which potently inhibits uptake and shows an ability to increase AEA concentrations and to reduce formalin-induced paw licking in vivo [90]. However, this compound is a potent FAAH inhibitor and can, in addition, interact with other serine hydrolases [91, 92], which limits its usefulness as a pharmacological tool.

With respect to the hydrolysis of AEA, the enzyme involved is FAAH, and several selective inhibitors of the enzyme have been described (review, see [16]). The two best characterized of the potent compounds are URB597 (3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate) and OL-135 (1-oxo-1[5-(2-pyridyl)-2-yl]-7-phenylheptane) [93, 94], both of which inhibit FAAH at nanamolar concentrations. URB597 acts by carbamylation of a key FAAH serine residue [95], although the relatively rapid (≤24 h) recovery of enzyme activity in the brain in vivo [96] is somewhat shorter than seen for irreversible inhibitors such as octylsulfonyl fluoride [97]. The second-order rate constant for the time-dependent inhibition of FAAH by URB597 is approximately eightfold lower at pH 6 than at pH 8 [98], which might be of importance in inflamed tissues, where the pH is reduced [99]. OL-135 acts as a competitive inhibitor of a truncated form of rat FAAH [100] and can protect the brain enzyme against inhibition by URB597 in vivo [101]. Both compounds are very selective for FAAH vs other cannabinoid targets [93, 94], and, although URB597 interacts with the serine hydrolase enzyme triacylglycerol hydrolase [94], it does not affect the activity of the enzyme [102].

In the case of these FAAH inhibitors, the question of selectivity is not vis-a-vis the compounds, rather the consequences of FAAH inhibition. Because FAAH has a wide substrate selectivity, inhibition of FAAH in vivo leads to increases in the concentration not only of AEA but also of other *N*-acylethanolamine substrates, such as palmitoylethanolamide and oleoylethanolamide, as well as other lipids such as the *N*-acyl taurines [94, 96, 103], which have a range of biological actions of their own. Palmitoylethanolamide, for example, has anti-inflammatory actions that can be mediated by peroxisome proliferator-activated receptor-α pathways (see [104, 105] and references therein), and recent data has indicated that the ability of SR144528 to block these actions is due to effects on peroxisome proliferator-activated receptor-α function rather than antagonism of CB_2 receptors [106]. Oleoylethanolamide can also interact with multiple targets, including TRPV1, peroxisome proliferator-activated receptor-α, and the orphan

receptor GPR119 [107–109], and *N*-arachidonoyltaurine can activate both TRPV1 and TRPV4 receptors [103]. Such a multiple response to FAAH inhibition means that an observed effect may not necessarily be due solely to an increase in AEA concentrations—indeed, in the case of the SR144528-sensitive effects of URB597 on inflamed tissue [110, 111], substrates like palmitoylethanolamide may be at least as important, and AEA may in fact detract from the net response by local activation of TRPV1 receptors (see [62, 112]).

2-AG is a substrate for FAAH [113], but in the brain, the enzyme monoacylglycerol lipase (MAGL) is probably more important [114]. Less is known about the pharmacology of this enzyme that can metabolise both 2-AG and 2-oleoylglycerol (a substrate often used in MAGL assays), but data is beginning to emerge in this respect. Thus, for example, the compound 3-(decylthio)-1,1,1-trifluoropropan-2-one at a concentration of 1 μM decreased (albeit modestly) the hydrolysis of 2-oleoylglycerol by cytosolic, but not membrane, fractions obtained from PC-3 prostate cancer cells and increased their 2-AG content [115]. The ability to distinguish pharmacologically the 2-oleoylglycerol hydrolysing activity in membrane and cytosolic fractions has been observed with other compounds as well [116]. Whether or not this reflects enzyme heterogeneity or is a consequence of the highly lipophilic nature of the compounds (and hence how they are presented to the enzymes in the different fractions) awaits elucidation.

A more potent inhibitor of 2-AG metabolism is the sulphydryl reagent *N*-arachidonoylmaleimide, which inhibited its hydrolysis by rat cerebellar membranes with an IC_{50} value of 14 nM after a preincubation phase of 30 min at 25°C [117]. Effects of this compound upon other aspects of cannabinoid signaling were not reported in this study. However, CB receptors, AEA synthesis and FAAH are known to be sensitive to sulphydryl reagents [118–120], and *N*-arachidonoylmaleimide is, thus, more useful as a tool to explore the properties of MAGL in vitro than in vivo.

Recently, the compound URB602 ([1,1′-biphenyl]-3-yl-carbamic acid, cyclohexyl ester) was reported to be a selective MAGL inhibitor, to potentiate baseline and ionomycin-induced 2-AG but not AEA levels in rat forebrain organotypic slice cultures and to enhance non-opioid stress-induced analgesia [121]. The compound was found to inhibit rat brain cytosolic 2-oleoylglycerol hydrolysis at recombinant rat brain MAGL with IC_{50} values of 28 and 75 μM, respectively, with no effect upon FAAH activity even at a concentration of 100 μM [121, 122]. Other authors, however, have not been able to demonstrate selectivity for MAGL vs FAAH [123]. A second compound, URB754 (6-methyl-2-[(4-methylphenyl)amino]-4H-3,1-benzoxazin-4-one), has also been described. This compound was identified in a screening library and was reported to inhibit recombinant rat brain MAGL with an IC_{50} value of 200 nM with little effect on FAAH or CB_1 receptors, to increase 2-AG levels in rat forebrain slice cultures and to potentiate depolarization-induced suppression of inhibition in hippocampal slices [122]. However, neither URB754 (100 μM) nor URB602 (1 mM) was found to affect the ability of either rat cerebellar membranes or brain homogenates to degrade 2-AG [124], see also [123], and URB754 at a concentration of 10 μM did not enhance the ability of 2-AG to stimulate [^{35}S]GTPγS binding in CB_1 receptor-rich regions of the rat brain [124], so the usefulness of these compounds is unclear.

The COX Connection

Throughout this article, a focus has been placed upon the selectivity of compounds with respect to other targets, both within and outside of the cannabinoid system. But the reverse, namely, that compounds with primary actions upon other systems can also interact with the endocannabinoid system, should also be considered. The most investigated in

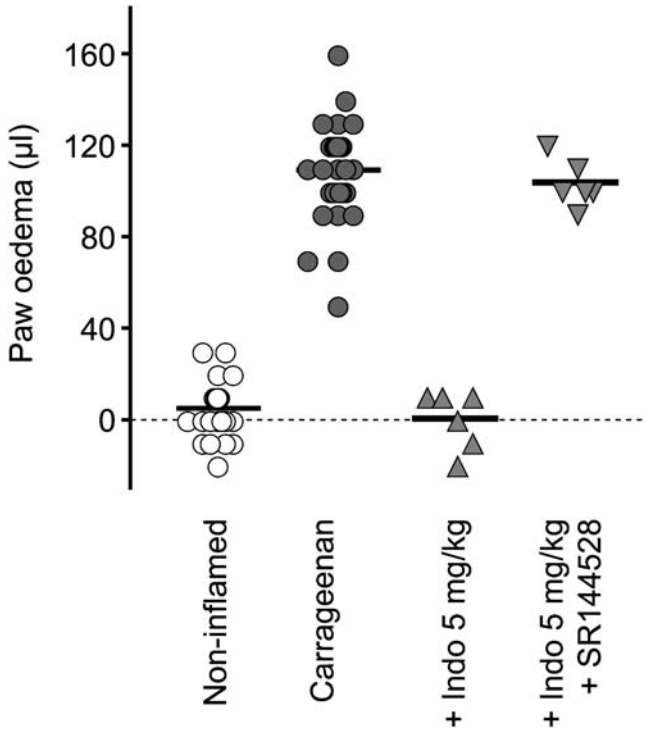

Fig. 2 The ability of indomethacin (5 mg/kg i.p.) to reduce carrageenan-induced paw edema in pentobarbital-treated mice is blocked by SR144528 (3 mg/kg i.p.). The figure is drawn from the original data of [110]. SR144528 and indomethacin were administered 40 and 30 min, respectively, before carrageenan, and the edema was measured 240 min after carrageenan administration. The data are the original values for Table 1 of [110]. One-way analysis of variance (ANOVA) of the complete data set (including the appropriate controls; Table 1 of [110]) indicated a significant effect of indomethacin on the paw edema, but not of indomethacin + SR144528

this respect are the non-steroidal anti-inflammatory drugs (NSAIDs). In brief,

- AEA and 2-AG are substrates for COX-2 (see [125]).
- Many NSAIDs, including ibuprofen, indomethacin, and flurbiprofen, inhibit the activity of FAAH, particularly at low pH (such as is seen in inflamed tissue) [126, 127]
- The effect of spinally administered indomethacin to mice in the formalin test of inflammatory pain is blocked by AM251 and is not seen in animals with a genetic deletion of the CB_1 receptor [128]. Flurbiprofen behaves in a similar manner [129]. In contrast, the efficacy of locally administered ibuprofen in the formalin test of inflammatory pain or its ability to reduce mechanical allodynia in hindpaws of rats rendered neuropathic by partial sciatic nerve ligation was not blocked by either AM251 or AM630 [130, 131]. On the other hand, the combination of AEA and ibuprofen did produce greater effects on both early and late phases of the formalin test than either compound per se. The effects of the combination on both phases was blocked by AM251 [130]. Neither ibuprofen or reficoxib changed levels of AEA, palmitoylethanolamide, or oleoylethanolamide per se, but the combination of AEA and either ibuprofen or reficoxib resulted in an increase in paw tissues of the levels of all three *N*-acylethanolamines [132].
- The ability of indomethacin to reduce carrageenan-induced edema in the mouse paw is blocked by SR144528 [110] (see Fig. 2), although this may be a peroxisome proliferator-activated receptor-α effect of this compound rather than an action upon CB_2 receptors [106].

The mechanism(s) responsible for these effects are as yet a matter of conjecture. Gühring et al. [128] and Ates et al. [129] suggested that inhibition of COX diverted the metabolism of arachidonic acid from prostaglandin to AEA synthesis. Holt et al. [110] suggested that a dual action of indomethacin to prevent the formation of inflammatory prostaglandins and to increase the levels of local endocannabinoids was required for anti-inflammatory effect in the mouse. Guindon et al. [131] hypothesized that the synergistic effects of ibuprofen and AEA in the formalin test reflected the ability of ibuprofen to inhibit FAAH and COX-2. Such suggestions, and indeed whether or not the endocannabinoid system contributes to the effects of NSAIDs in man, await elucidation.

Paracetamol (acetaminophen), although not an NSAID, also interacts with the endocannabinoid system inasmuch as its analgesic effects in the hot plate test (but not in the phenyl-*p*-quinone pain model) are blocked by rimonabant or AM251 [133, 134] and that it can be metabolized to AM404 by a mechanism dependent upon FAAH [84], but it does not have direct effects on the ability of FAAH to metabolize AEA in vitro [126]. Given that rimonabant is now clinically available for the treatment of obesity, information on the interaction (or lack thereof) between this compound and paracetamol in man may appear in due course.

Conclusions

The aim of this review has been to present the most commonly used compounds that have been used to probe the functions of the endocannabinoid system in the body. A deliberate focus has been made on the issue of the selectivities of the compounds, which has been exemplified with the compounds WIN55212,2 and AM404. Nonetheless, in the relatively short time since the cannabinoid receptors were cloned, the availability of useful compounds has been a key factor in furthering our understanding of the endocannabinoid system. Much remains to be done: In terms of the endocannabinoid system, inhibitors of the synthesis of AEA are lacking and the functional roles of, for example, MAGL and the roles played by COX-2-derived metabolites of AEA and 2-AG are only beginning to be investigated. The cannabis plant itself remains full of secrets, as exemplified by the recent finding that Δ^9-tetrahydrocannabivarin is an antagonist at CB_1 receptors [135]. Finally, the discovery of AEA-related endogenous, biologically active molecules, such as arachidonoylglycine [136] and arachidonoylserine [137], that have important actions of their own places the endocannabinoids as but one player in a team of different biologically active lipids. Clearly, the pharmacology of the endocannabinoid system and of related bioactive lipids is still at an early stage.

Note added in proof: Recently, Palomäki et al. (J Neurochem, 101 [2007] 972–981) reported that tetrahydrolipstatin antagonizes CB_1 receptors, which may confound interpretation of its reported effects upon retrograde signalling. The issue with URB754, on the other hand, has been clarified: the MAGL inhibitory properties reported in [122] were later found by those authors to be due to a mercuric contaminant (Makara et al., Nature Neurosci 10 [2007] 134).

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References

1. Zias J, Stark H, Seligman J, Levy R, Werker E, Breuer A, Mechoulam R (1993) Early medical use of cannabis. Nature 363:215

2. Gaoni Y, Mechoulam R (1964) Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 86:1646–1647
3. Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3:771–784
4. Jonsson K-O, Holt S, Fowler CJ (2006) The endocannabinoid system: current pharmacological research and therapeutic possibilities. Basic Clin Pharmacol Toxicol 98:124–134
5. Fowler CJ (2005) Pharmacological properties and therapeutic possibilities for drugs acting upon endocannabinoid receptors. Curr Drug Targets CNS Neurol Disord 4:685–696
6. Mackie K (2006) Cannabinoid receptors as therapeutic targets. Annu Rev Pharmacol Toxicol 46:101–122
7. Begg M, Pacher P, Bátkai S, Osei-Hyiaman D, Offertáler L, Mo FM, Liu J, Kunos G (2005) Evidence for novel cannabinoid receptors. Pharmacol Ther 106:133–145
8. Baker D, Pryce G, Davies WL, Hiley CR (2006) *In silico* patent searching reveals a new cannabinoid receptor. Trends Pharmacol Sci 27:1–4
9. Barann M, Molderings G, Brüss M, Bönisch H, Urban BW, Göthert M (2002) Direct inhibition by cannabinoids of human 5-HT_{3A} receptors: probable involvement of an allosteric modulatory site. Br J Pharmacol 137:589–596
10. Zygmunt PM, Petersson J, Andersson DA, Chuang H-h, Sørgård M, Di Marzo V, Julius D, Högestätt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457
11. Walton RP, Martin LF, Keller JH (1938) The relative activity of various purified products obtained from American grown hashish. J Pharmacol Exp Ther 62:239–251
12. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International union of pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161–202
13. Salo OMH, Savinainen JR, Parkkari T, Nevalainen T, Lahtela-Kakkonen ML, Gynther J, Laitinen JT, Järvinen T, Poso A (2006) 3D-QSAR studies on cannabinoid CB1 receptor agonists: G-protein activation as biological data. J Med Chem 49:554–566
14. Martin BR, Wiley JL, Beletskaya I, Sim-Selley LJ, Smith FL, Dewey WL, Cottney J, Adams J, Baker J, Hill D, Saha B, Zerkowski J, Mahadevan A, Razdan RK (2006) Pharmacological characterization of novel water-soluble cannabinoids. J Pharmacol Exp Ther 318:1230–1239
15. Lange JHM, Kruse CG (2004) Recent advances in CB_1 cannabinoid receptor antagonists. Curr Opin Drug Discov Dev 7:498–506
16. Lambert DM, Fowler CJ (2005) The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. J Med Chem 48:5059–5087
17. Padgett LW (2005) Recent developments in cannabinoid ligands. Life Sci 77:1767–1798
18. Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, McLean A, McIntosh L, Goodwin G, Walker G, Westwood P, Marrs J, Thomson F, Cowley P, Christopoulos A, Pertwee RG, Ross RA (2005) Allosteric modulation of the cannabinoid CB_1 receptor. Mol Pharmacol 68:1484–1495
19. Pertwee RG (2001) Cannabinoid receptors and pain. Prog Neurobiol 63:569–611
20. Diana MA, Marty A (2004) Endocannabinoid-mediated short-term synaptic plasticitiy: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). Br J Pharmacol 142:9–19
21. Park B, McPartland JM, Glass M (2004) Cannabis, cannabinoids and reproduction. Prostaglandins Leukot Essent Fat Acids 70:189–197
22. Croxford JL, Yamamura T (2005) Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? J Neuroimmunol 166:3–18
23. De Vries TJ, Schoffelmeer ANM (2005) Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci 26:420–426
24. Kirkham TC (2005) Endocannabinoids in the regulation of appetite and body weight. Behav Pharmacol 16:297–313
25. Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat Rev Immunol 5:400–411
26. Rang HP, Dale MM, Ritter JM, Moore PK (2003) Pharmacology, 5th edn. Churchill Livingstone, Edinburgh, pp 15–17
27. Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB_1 receptors. Life Sci 76:1307–1324
28. Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand J-P, Le Fur G, Casellas P (1997) A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J Biol Chem 272:22330–22339
29. Vásquez C, Lewis DL (1999) The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. J Neurosci 19:9271–9280
30. Bouaboula M, Desnoyer N, Carayon P, Combes T, Casellas P (1999) G_i protein modulation induced by a selective inverse agonist for the peripheral cannabinoid receptor CB2: implication for intracellular signalization cross-regulation. Mol Pharmacol 55:473–480
31. Mukhopadhyay S, Howlett AC (2001) CB_1 receptor–G protein association. Subtype selectivity is determined by distinct intracellular domains. Eur J Biochem 268:499–505
32. Mukhopadhyay S, Howlett AC (2005) Chemically distinct ligands promote differential CB_1 cannabinoid receptor–G_i protein interactions. Mol Pharmacol 67:2016–2024
33. Bonhaus DW, Chang LK, Kwan J, Martin GR (1998) Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. J Pharmacol Exp Ther 287:884–888
34. Shoemaker JL, Ruckle MB, Mayeux PR, Prather PL (2005) Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors. J Pharmacol Exp Ther 315:828–838
35. Yao BB, Mukherjee S, Fan Y, Garrison TR, Daza AV, Grayson GK, Hooker BA, Dart MJ, Sullivan JP, Meyer MD (2006) *In vitro* pharmacological characterization of AM1241: a protean agonist at the cannabinoid CB_2 receptor? Br J Pharmacol 149:145–154
36. Kenakin T (2001) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. FASEB J 15:598–611
37. Shim J-Y, Howlett AC (2006) WIN55,212-2 docking to the CB_1 cannabinoid receptor and multiple pathways for conformational induction. J Chem Inf Model 46:1286–1300
38. Shen C-P, Xiao JC, Armstrong H, Hagmann W, Fong TM (2006) F200A substitution in the third transmembrane helix of human cannabinoid CB_1 receptor converts AM2233 from receptor agonist to inverse agonist. Eur J Pharmacol 531:41–46
39. Luk T, Jin W, Zvonok A, Lu D, Lin X-Z, Chavkin C, Makriyannis A, Mackie K (2004) Identification of a potent and highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. Br J Pharmacol 142:495–500
40. Hilaret S, Bouaboula M, Carrière D, Le Fur G, Casellas P (2003) Hypersensitization of the orexin 1 receptor by the CB1 receptor. Evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. J Biol Chem 278:23731–23737
41. Mackie K (2005) Cannabinoid receptor homo- and heterodimerization. Life Sci 77:1667–1673
42. Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR (1992) Aminoalkylindole analogs: cannabimimetic activity of a class of

compounds structurally distinct from delta 9-tetrahydrocannabinol. J Pharmacol Exp Ther 263:1118–1126
43. Savinainen JR, Kokkola T, Salo OMH, Poso A, Järvinen T, Laitinen JT (2005) Identification of WIN55212-3 as a competitive neutral antagonist of the human cannabinoid CB_2 receptor. Br J Pharmacol 145:636–645
44. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, Harvey-White J, Mackie K, Offertáler L, Wang L, Kunos G (2005) Endocannabinoid activation at hepatic CB_1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 115:1298–1305
45. Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci 26:2991–3001
46. Bloom AS, Edgemond WS, Moldvan JC (1997) Nonclassical and endogenous cannabinoids: effects on the ordering of brain membranes. Neurochem Res 22:563–568
47. Sagan S, Venance L, Torrens Y, Cordier J, Glowinski J, Giaume C (1999) Anandamide and WIN 55212-2 inhibit cyclic AMP formation through G-protein coupled receptors distinct from CB1 cannabinoid receptors in cultured astrocytes. Eur J Neurosci 11:691–699
48. Breivogel CS, Griffin G, Di Marzo V, Martin BR (2001) Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 60:155–163
49. Hájos N, Ledent C, Freund TF (2001) Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 106:1–4
50. Hoffman AF, Macgill AM, Smith D, Oz M, Lupica CR (2005) Species and strain differences in the expression of a novel glutamate-modulating cannabinoid receptor in the rodent hippocampus. Eur J Neurosci 22:2387–2391
51. Facchinetti F, Del Giuduce E, Furegato S, Passarotto M, Leon A (2003) Cannabinoids ablate release of TNFα in rat microglial cells stimulated with lypopolysaccharide. Glia 41:161–168
52. Nieri P, Greco R, Adinolfi B, Breschi MC, Martinotti E, Nannetti C, Podestà A (2003) CB_1-and CB_2-cannabinoid receptor-independent lipolysis induced by WIN 55,212-2 in male in rat adipocytes. Naunyn–Schmiedeberg's Arch Pharmacol 368:352–359
53. Köfalvi A, Vizi ES, Ledent C, Sperlágh B (2003) Cannabinoids inhibit the release of [^{3}H]glutamate from rodent hippocampal synaptosomes via a novel CB_1 receptor-independent action. Eur J Neurosci 18:1973–1978
54. Köfalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA, Sperlágh B (2005) Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J Neurosci 25:2874–2884
55. Nicholson RA, Liao C, Zheng J, David LS, Coyne L, Errington AC, Singh G, Lees G (2003) Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. Brain Res 978:194–204
56. Pistis M, Perra S, Pillolla G, Melis M, Gessa GL, Muntoni AL (2004) Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. Neuropharmacology 46:115–125
57. Price TJ, Patwardhan A, Akopian AN, Hargreaves KM, Flores CM (2004) Cannabinoid receptor-independent actions of the aminoalkylindole WIN 55,212-2 on trigeminal sensory neurons. Br J Pharmacol 142:257–266
58. Steffens M, Feuerstein TJ (2004) Receptor-independent depression of DA and 5-HT uptake by cannabinoids in rat neocortex—involvement of Na^+/K^+-ATPase. Neurochem Int 44:529–538
59. Curran NM, Griffin BD, O'Toole D, Brady KJ, Fitzgerald SN, Moynagh PN (2005) The synthetic cannabinoid *R*(+) WIN55,212-2 inhibits the interleukin-1 signaling pathway in human astrocytes in a cannabinoid-receptor-independent manner. J Biol Chem 280:35797–35806
60. Nilsson O, Fowler CJ, Jacobsson SOP (2006) The cannabinoid agonist WIN 55,212-2 inhibits TNF-α-induced neutrophil transmigration across ECV304 cells. Eur J Pharmacol 547:165–173
61. Ahluwalia J, Urban L, Bevan S, Nagy I (2003) Anandamide regulates neuropeptide release from capsaicin-sensitive primary sensory neurons by activating both the cannabinoid 1 receptor and the vanilloid receptor 1 *in vitro*. Eur J Neurosci 17:2611–2618
62. Singh Tahim A, Sántha P, Nagy I (2005) Inflammatory mediators convert anandamide into a potent activator of the vanilloid type 1 transient receptor potential receptor in nociceptive primary sensory neurons. Neuroscience 136:539–548
63. Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-*N*-acyl ethanolamine and a role for α/ß-hydrolase 4 in this pathway. J Biol Chem 281: 26465–26472
64. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim H-Y, Kunos G (2006) A biosynthetic pathway for anandamide. Proc Natl Acad Sci USA 103:13345–13350
65. Melis M, Perra S, Muntoni AL, Pillolla G, Lutz B, Marsicano G, Di Marzo V, Gessa GL, Pistis M (2004) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci 24:10707–10715
66. Safo PK, Regehr WG (2005) Endocannabinoids control the induction of cerebellar LTD. Neuron 48:647–659
67. Bisogno T, Cascio MG, Saha B, Mahadevan A, Urbani P, Minassi A, Appendino G, Saturnino C, Martin B, Razdan R, Di Marzo V (2006) Development of the first potent and specific inhibitors of endocannabinoid biosynthesis. Biochim Biophys Acta 1761:205–212
68. Ligresti A, Morera E, Van Der Stelt MM, Monory K, Lutz B, Ortar G, Di Marzo V (2004) Further evidence for the existence of a specific process for the membrane transport of anandamide. Biochem J 380:265–272
69. Ronesi J, Gerdeman GL, Lovinger DM (2004) Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci 24:1673–1679
70. Straiker A, Mackie K (2005) Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. J Physiol 569:501–517
71. Karlsson M, Påhlsson C, Fowler CJ (2004) Reversible, temperature-dependent, and AM404-inhibitable adsorption of anandamide to cell culture wells as a confounding factor in release experiments. Eur J Pharm Sci 22:181–189
72. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz J-C, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691
73. Hillard CJ, Edgemond WS, Jarrahian A, Campbell WB (1997) Accumulation of *N*-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. J Neurochem 69:631–638
74. Kaczocha M, Hermann A, Glaser ST, Bojesen IN, Deutsch DG (2006) Anandamide uptake is consistent with rate-limited diffusion and is regulated by the degree of its hydrolysis by FAAH. J Biol Chem 281:9066–9075
75. Hermann A, Kaczocha M, Deutsch DG (2006) 2-Arachidonoylglycerol (2-AG) membrane transport: history and outlook. AAPS J 8:E409–E412
76. Fowler CJ (2006) The cannabinoid system and its pharmacological manipulation—a review, with emphasis upon the uptake and hydrolysis of anandamide. Fundam Clin Pharmacol 20:549–562

77. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277:1094–1097
78. De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V (2000) Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. FEBS Lett 483:52–56
79. López-Rodríguez ML, Viso A, Ortega-Gutiérrez S, Fowler CJ, Tiger G, de Lago E, Fernández-Ruiz J, Ramos JA (2003) Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors: comparison with effects upon fatty acid amidohydrolase. J Med Chem 46:1512–1522
80. Ortar G, Ligresti A, De Petrocellis L, Morera E, Di Marzo V (2003) Novel selective and metabolically stable inhibitors of anandamide cellular uptake. Biochem Pharmacol 65:1473–1481
81. Khanolkar AD, Abadji V, Lin S, Hill AG, Taha G, Abouzid K, Meng Z, Fan P, Makriyannis A (1996) Head group analogs of arachidonoylethanolamide, the endogenous cannabinoid ligand. J Med Chem 39:4515–4519
82. Zygmunt PM, Chuang H-h, Movahed P, Julius D, Högestätt ED (2000) The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur J Pharmacol 396:39–42
83. Kelley BG, Thayer SA (2004) Anandamide transport inhibitor AM404 and structurally related compounds inhibit synaptic transmission between rat hippocampal neurons in culture independent of cannabinoid CB_1 receptors. Eur J Pharmacol 496:33–39
84. Högestätt ED, Jönsson BAG, Ermund A, Andersson DA, Björk H, Alexander JP, Cravatt BF, Basbaum AI, Zygmunt PM (2005) Conversion of acetaminophen to the bioactive *N*-acyl phenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. J Biol Chem 280:31405–31412
85. Chen W-C, Huang J-K, Cheng J-S, Tsai JC-R, Chiang A-J, Chou K-J, Liu C-P, Jan C-R (2001) AM-404 elevates renal intracellular Ca^{2+}, questioning its selectivity as a pharmacological tool for investigating the anandamide transporter. J Pharmacol Toxicol Methods 45:195–198
86. De Lago E, Gustafsson SB, Fernández-Ruiz J, Nilsson J, Jacobsson SOP, Fowler CJ (2006) Acyl-based anandamide uptake inhibitors cause rapid toxicity to C6 glioma cells at pharmacologically relevant concentrations. J Neurochem 99:677–688
87. La Rana G, Russo R, Campolongo P, Bortolato M, Mangieri RA, Cuomo V, Iacono A, Raso GM, Meli R, Piomelli D, Calignano A (2006) Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404 [*N*-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide]. J Pharmacol Exp Ther 317:1365–1371
88. Lastres-Becker I, de Miguel R, De Petrocellis L, Makriyannis A, Di Marzo V, Fernández-Ruiz J (2003) Compounds acting at the endocanabinoid and/or endovanilloid systems reduce hyperkinesia in a rat model of Huntington's disease. J Neurochem 84:1097–1109
89. Fowler CJ, Holt S, Nilsson O, Jonsson K-O, Tiger G, Jacobsson SOP (2005) The endocannabinoid signaling system: pharmacological and therapeutic aspects. Pharmacol Biochem Behav 81:248–262
90. Moore SA, Nomikos GG, Dickason-Chesterfield AK, Schober DA, Schaus JM, Ying B-P, Xu T-CV, Phebus L, Simmons RMA, Li D, Iyengar S, Felder CC (2005) Identification of a high-affinity binding site involved in the transport of endocannabinoids. Proc Natl Acad Sci U S A 102:17852–17857
91. Dickason-Chesterfield AK, Kidd SR, Moore SA, Schaus JM, Liu B, Nomikos GG, Felder CC (2006) Pharmacological characterization of endocannabinoid transport and fatty acid amide hydrolase inhibitors. Cell Mol Neurobiol 26:407–423
92. Alexander JP, Cravatt BF (2006) The putative endocannabinoid transport blocker LY2183240 is a potent inhibitor of FAAH and several other brain serine hydrolases. J Am Chem Soc 128:9699–9704
93. Kathuria S, Gaetani S, Fegley D, Valiño F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nature Med 9:76–81
94. Lichtman AH, Leung D, Shelton CC, Saghatelian A, Hardouin C, Boger DL, Cravatt BF (2004) Reversible inhibitors of fatty acid amide hydrolase that promote analgesia: evidence for an unprecedented combination of potency and selectivity. J Pharmacol Exp Ther 311:441–448
95. Alexander JP, Cravatt BF (2005) Mechanisms of carbamate inactivation of FAAH: implications for the design of covalent inhibitors and in vivo functional probes for enzymes. Chem Biol 12:1179–1187
96. Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A, Piomelli D (2004) Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc Natl Acad Sci USA 101:8756–8761
97. Quistad GB, Sparks SE, Segall Y, Nomura DK, Casida JE (2002) Selective inhibitors of fatty acid amide hydrolase relative to neuropathy target esterase and acetylcholinesterase: toxicological implications. Toxicol Appl Pharm 179:57–63
98. Paylor B, Holt S, Fowler CJ (2006) The potency of the fatty acid amide hydrolase inhibitor URB597 is dependent upon the assay pH. Pharmacol Res 54:481–485. Corrigendum published in Pharmacol Res 55 [2007] 80
99. Häbler C (1929) Über den K- und Ca-gehalt von eiter und exsudaten und seine beziehungen zum entzündungsschmerz. Klin Wochenschr 8:1569–1572
100. Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, Cheng H, Hwang I, Hedrick MP, Leung D, Acevedo O, Guimarães CRW, Jorgensen WL, Cravatt BF (2005) Discovery of a potent, selective, and efficacious class of reversible α-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. J Med Chem 48:1849–1856
101. Chang L, Luo L, Palmer JA, Sutton S, Wilson SJ, Barbier AJ, Breitenbucher JG, Chaplan SR, Webb M (2006) Inhibition of fatty acid amide hydrolase produces analgesia by multiple mechanisms. Br J Pharmacol 148:102–113
102. Clapper JR, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2006) The fatty-acid amide hydrolase inhibitor URB597 does not affect triacylglycerol hydrolysis in rat tissues. Pharmacol Res 54:341–344
103. Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF (2006) A FAAH-regulated class of *N*-acyl taurines that activates TRP ion channels. Biochemistry 45:9008–9015
104. Farquhar-Smith W, Rice ASC (2003) A novel neuroimmune mechanism in cannabinoid-mediated attenuation of nerve growth factor-induced hyperalgesia. Anesthesiology 99:1391–1401
105. Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, Piomelli D (2005) The nuclear receptor peroxisome proliferator-activated receptor-α mediates the anti-inflammatory actions of palmitoylethanolamide. Mol Pharmacol 67:15–19
106. Lo Verme J, Russo R, La Rana G, Fu J, Farthing JN, Mattace-Raso G, Meli R, Hohmann A, Calignano A, Piomelli D (2006) Rapid broad-spectrum analgesia through activation of peroxisome proliferator-activated receptor-α. J Pharmacol Exp Ther 319:1051–1061

107. Ahern GP (2003) Activation of TRPV1 by the satiety factor oleoylethanolamide. J Biol Chem 278:30429–30434
108. Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez de Fonseca F, Rosengarth A, Luecke H, Di Giacomo B, Tarzia G, Piomelli D (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature 425:90–93
109. Overton HA, Babbs AJ, Doel SM, Fyfe MCT, Gardner LS, Griffin G, Jackson HC, Procter MJ, Rasamison CM, Tang-Christensen M, Widdowson PS, Williams GM, Reynet C (2006) Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metab 3:167–175
110. Holt S, Comelli F, Costa B, Fowler CJ (2005) Inhibitors of fatty acid amide hydrolase reduce carrageenan-induced hind paw inflammation in pentobarbital-treated mice: comparison with indomethacin and possible involvement of cannabinoid receptors. Br J Pharmacol 146:467–476
111. Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW (2006) Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. Br J Pharmacol 147:281–288
112. Dinis P, Charrua A, Avelino A, Yaqoob M, Bevan S, Nagy I, Cruz F (2004) Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 24:11253–11263
113. Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S (1998) Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. FEBS Letts 422:69–73
114. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 99:10819–10824
115. Nithipatikom K, Endsley MP, Isbell MA, Wheelock CE, Hammock BD, Campbell WB (2005) A new class of inhibitors of 2-arachidonoylglycerol hydrolysis and invasion of prostate cancer cells. Biochem Biophys Res Commun 332:1028–1033
116. Vandevoorde S, Saha B, Mahadevan A, Razdan RK, Pertwee RG, Martin BR, Fowler CJ (2005) Influence of the degree of unsaturation of the acyl side chain upon the interaction of analogues of 1-arachidonoylglycerol with monoacylglycerol lipase and fatty acid amide hydrolase. Biochem Biophys Res Commun 337:104–109
117. Saario SM, Salo OMH, Nevalainen T, Poso A, Laitinen JT, Järvinen T, Niemi R (2005) Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. Chem Biol 12:649–656
118. Lu R, Hubbard JR, Martin BR, Kalimi MY (1993) Roles of sulfhydryl and disulfide groups in the binding of CP-55,940 to rat brain cannabinoid receptor. Mol Cell Biochem 121:119–126
119. Sasaki T, Chang MCJ (1997) N-Arachidonoylethanolamine (anandamide) formation from *N*-arachidonoylphosphatidolethanolamine in rat brain membranes. Life Sci 61:1803–1810
120. Schmid PC, Zuzarte-Augustin ML, Schmid HHO (1985) Properties of rat liver *N*-acylethanolamine amidohydrolase. J Biol Chem 260:14145–14149
121. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2005) An endocannabinoid mechanism for stress-induced analgesia. Nature 435:1108–1112
122. Makara JK, Mor M, Fegley D, Szabó SI, Kathuria S, Astarita G, Duranti A, Tontini A, Tarzia G, Rivara S, Freund TF, Piomelli D (2005) Selective inhibition of 2-AG enhances endocannabinoid signaling in hippocampus. Nat Neurosci 8:1139–1141
123. Vandevoorde S, Jonsson K-O, Labar G, Persson E, Lambert DM, Fowler CJ (2007) Lack of selectivity of URB602 for 2-oleoylglycerol compared to anandamide hydrolysis *in vitro*. Br J Pharmacol 150:186–191
124. Saario SM, Palomäki V, Lehtonen M, Nevalainen T, Järvinen T, Laitinen JT (2006) URB754 has no effect on the hydrolysis or signalling capacity of 2-AG in the rat brain. Chem Biol 13:811–814
125. Kozak KR, Marnett LJ (2002) Oxidative metabolism of endocannabinoids. Prostaglandins Leukot Essent Fatty Acids 66:211–220
126. Fowler CJ, Tiger G, Stenström A (1997) Ibuprofen inhibits rat brain deamidation of anandamide at pharmacologically relevant concentrations. Mode of inhibition and structure-activity relationship. J Pharmacol Exp Ther 283:729–734
127. Fowler CJ, Holt S, Tiger G (2003) Acidic non-steroidal anti-inflammatory drugs inhibit rat brain fatty acid amide hydrolase in a pH-dependent manner. J Enzym Inhib Med Chem 18:55–58
128. Gühring H, Hamza M, Sergejeva M, Ates M, Kotalla CE, Ledent C, Brune K (2002) A role for endocannabinoids in indomethacin-induced spinal antinociception. Eur J Pharmacol 454:153–163
129. Ates M, Hamza M, Seidel K, Kotalla CE, Ledent C, Gühring H (2003) Intrathecally applied flurbiprofen produces an endocannabinoid-dependent antinociception in the rat formalin test. Eur J Neurosci 17:597–604
130. Guindon J, Beaulieu P (2006) Antihyperalgesic effects of local injections of anandamide, ibuprofen, rofecoxib ands their combinations in a model of neuropathic pain. Neuropharmacology 50:814–823
131. Guindon J, De Léan A, Beaulieu P (2006) Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. Pain 121:85–93
132. Guindon J, LoVerme J, De Léan A, Piomelli D, Beaulieu P (2006) Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: a role for endogenous fatty-acid ethanolamides? Eur J Pharmacol 550:68–77
133. Ottani A, Leone S, Sandrini M, Ferrari A, Bertolini A (2006) The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB_1 receptors. Eur J Pharmacol 531:280–281
134. Haller VL, Cichewicz DL, Welch SP (2006) Non-cannabinoid CB_1, non-cannabinoid CB_2 antinociceptive effects of several novel compounds in the PPQ stretch test in mice. Eur J Pharmacol 546:60–68
135. Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA, Pertwee RG (2005) Evidence that the plant cannabinoid Δ^9-tetrahydrocannabivarin is a cannabinoid CB_1 and CB_2 receptor antagonist. Br J Pharmacol 146:917–926
136. Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE, Sivakumar R, Coop A, Maeda DY, De Petrocellis L, Burstein S, Di Marzo V, Walker JM (2001) Identification of a new class of molecules, the arachidonoyl amino acids, and characterization of one member that inhibits pain. J Biol Chem 276:42639–42644
137. Milman G, Maor Y, Abu-Lafi S, Horowitz M, Gallily R, Batkai S, Mo FM, Offertaler L, Pacher P, Kunos G, Mechoulam R (2006) *N*-arachidonoyl *L*-serine, an endocannabinoid-like brain constituent with vasodilatory properties. Proc Natl Acad Sci USA 103:2428–2433