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In vivo pharmacology of endocannabinoids and their metabolic inhibitors:

therapeutic implications in Parkinson's disease and abuse liability

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Abstract

This review focuses on the behavioral pharmacology of endogenous cannabinoids (endocannabinoids) and indirect-acting cannabinoid agonists that elevate endocannabinoid tone by inhibiting the activity of metabolic enzymes. Similarities and differences between prototype cannabinoid agonists, endocannabinoids and inhibitors of endocannabinoid metabolism are discussed in the context of endocannabinoid pharmacokinetics in vivo. The distribution and function of cannabinoid and non-CB₁/CB₂ receptors are also covered, with emphasis on their role in disorders characterized by dopamine dysfunction, such as drug abuse and Parkinson's disease. Finally, evidence is presented to suggest that FAAH inhibitors lack the abuse liability associated with CB₁ agonists, although they may modify the addictive properties of other drugs, such as alcohol.

1. Endocannabinoid ligands and receptors

The endocannabinoid system consists of a family of lipid signaling molecules (endocannabinoids), their biosynthetic and metabolic enzymes and associated cannabinoid receptors. Recent studies indicate that endocannabinoids can activate multiple receptor targets, including not only metabotropic (i.e., CB₁ and CB₂) but also ionotropic and nuclear receptors. This chapter focuses on conventional cannabinoid and non-CB₁/CB₂ receptors in the central nervous system (CNS) and on the enzymes responsible for endocannabinoid degradation: fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The pharmacological and molecular mechanisms of endocannabinoid re-uptake, and the biological effects resulting from activation of cannabinoid-related targets outside the CNS, have been covered by other reviews^{1,2} and will not be discussed here.

1.1 Endocannabinoid receptors

To date, two G protein-coupled cannabinoid receptor subtypes - CB₁ and CB₂ - have been cloned³. Within the CNS, CB₁ receptors are mainly expressed in the basal ganglia, cerebellum, hippocampus, and cortex⁴⁻⁷, and their activation has been associated with most of the psychotropic and behavioral actions of cannabinoid drugs. By contrast, CB₂ receptors are primarily localized in cells involved in immune and inflammatory responses⁸⁻¹⁰. CB₂ receptors are also expressed in the cerebellum and brain stem^{11,12} and they modulate the mobility and

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function of microglial cells *in vitro*¹³ and *in vivo*¹⁴. Both receptor subtypes are $G_{i/o}$ -coupled and, when activated, they initiate signaling events typically associated with this class of G proteins, e.g. inhibition of cAMP accumulation and cAMP-dependent protein kinase (PKA)¹⁵. Noteworthy, CB₁ receptors are also constitutively active in the absence of exogenously applied agonists¹⁶ and distinct cannabinoid ligands have been shown to promote CB₁ coupling to different G_i isoforms¹⁷. CB₁ receptors may also couple to G_s proteins^{18,19} and form heterodimers with dopamine D₂ and mu-opioid receptors^{20,21}. Agonist-dependent activation of different signaling pathways has been also described for CB₂ receptors²².

Stimulation of CB₁ receptors inhibits N and P/Q-type voltage-gated Ca²⁺ channels²³⁻²⁶ and M-type K⁺ channels²⁷ and activates A-type and inwardly rectifying K⁺ currents²⁸, which have been implicated in the CB₁-mediated depression of GABA²⁹⁻³¹ and glutamate release³².

Consistent with their proposed modulatory role of inhibitory and excitatory neurotransmission, CB₁ receptors are located presynaptically on GABAergic neurons³³ and interneurons³⁴⁻³⁶ and on glutamatergic terminals^{32,37}.

CB₁ expression and activity is regulated via multiple mechanisms. In particular, extracellular signal-regulated kinase (ERK) and focal adhesion kinase (FAK) have been shown to affect CB₁ gene expression in neurons and to participate in changes in synaptic plasticity observed after administration of cannabinoid agonists³⁸.

The development of CB₁ and CB₂ knockout mice on different backgrounds (i.e., CD1, C57BL)³⁹⁻⁴², and of mutant mice lacking the CB₁ receptors in neuronal subpopulations^{34,43} has improved our understanding of the biological roles played by these receptors *in vivo* and showed that some of the effects of cannabinoid agonists persist after the ablation of CB₁ and CB₂ genes (for review see [44]). These non-CB₁/CB₂ targets include other G protein-coupled receptors (GPCR), ion channels (i.e., TRPV receptors) and nuclear receptors (i.e., PPAR).

Non-CB₁/CB₂ receptors—In adult CB₁ knockout mice, the observation that non-selective cannabinoids WIN55212-2 and CP55940 reduce excitatory, but not GABAergic, currents in the CA1 field of the hippocampus^{45,46} provided the first evidence for the existence of a cannabinoid site in the brain (also called “CB₃” or “WIN receptor”) that is distinct from CB₁, sensitive to pertussin toxin (PTX) and blocked by the cannabinoid antagonist SR141716A (rimonabant) - but not by its analog AM251 - and the TRPV1 antagonist capsazepine⁴⁵. Recent evidence, however, points to the CB₁ rather than the “CB₃” as the major cannabinoid receptor at the excitatory pre-synaptic sites of the hippocampus and cerebellum⁴⁷.

A G-protein-coupled cannabinoid site (the “abnormal-cannabidiol” receptor), which is insensitive to either capsazepine or WIN55212-2, has been identified in the vascular endothelium⁴⁸.

In 2001, a patent from GlaxoSmithKline reported the first association between cannabinoids and GPR55, a cloned orphan receptor of the purinergic subfamily⁴⁹, activated by rimonabant and AM251 and distinct from the abnormal-cannabidiol receptor^{44,49,50}. In 2004, a patent from AstraZeneca reported that several cannabinoid agonists and antagonists, including CP55940, rimonabant, anandamide (AEA), 2-arachidonoyl glycerol (2-AG) and ⁹Δ-THC, but not WIN55,212-2, bind to HEK293T cell membranes expressing GPR55 with EC₅₀ values in the low nanomolar range. These results were confirmed by two groups in GPR55-deficient mice^{50,51}, but not by Lauckner et al., which failed to find any stimulatory activity for 2-AG and CP55940 in HEK293 cells⁵².

GPR55 is insensitive to PTX, appears to signal via G_{αq} and phospholipase C beta (PLCβ) and requires G_{α12}, the GTPase RhoA and intact actin cytoskeleton to induce Ca²⁺ release from

intracellular stores⁵². Other signaling mechanisms, either G-protein dependent or independent have been also suggested⁵³. The low amino acid sequence homology shared with the cloned CB₁ and CB₂ receptors (13.5% and 14.4%, respectively) suggest that GPR55 is a receptor with a binding site for cannabinoid ligands, possibly arisen by convergent evolution towards these signaling molecules⁵⁴. Within the CNS, GPR55 mRNA is expressed in the caudate-putamen and, to a lesser extent, in hippocampus, thalamus and cerebellum⁴⁹. However, these data have not been validated in brain tissue from GPR55 null mice. The functional significance of GPR55 in the CNS remains unclear and relies upon the development of potent and selective ligands. The decreased response of GPR55^{-/-} mice to inflammatory and/or nociceptive stimuli⁵⁵, and the high expression of GPR55 in large-diameter DRG neurons⁵² suggest a possible role in pain signaling.

TRP channels—The transient receptor potential (TRP) receptors are ligand-gated ion channels that generate a cation inward flow upon activation⁵⁶. Experimental evidence indicates that cannabinoids and endocannabinoids directly interact with at least five distinct TRP channels, among which the vanilloid receptor VR1 (TRPV1)⁵⁷ emerges as the best-characterized ‘ionotropic’ cannabinoid receptor⁵⁸.

TRPV1 receptors are highly expressed in sensory nerves, where they participate in the processing of inflammatory and thermal pain⁵⁹. Within the brain, they are present in the striatum, globus pallidus and substantia nigra⁶⁰⁻⁶². In the latter region, they affect the excitability of dopamine neurons by modulating excitatory inputs from glutamatergic terminals⁶³. In vivo, activation of TRPV1 has been shown to have fear-promoting effects⁶⁴ and mediate the AEA ability to disrupt behavioral tasks measuring attention in rats⁶⁵. On the other hand, pharmacological blockade of TRPV1 unmasked the anti-dyskinetic effect of the FAAH inhibitor URB597 in a rat model of Parkinson’s disease⁶⁶ (see below).

Among the endocannabinoids, AEA can activate TRPV1^{67,68} and inhibit TRPM8 receptors⁶⁹, respectively. AEA can also indirectly activate TRPV4 channels via epoxyeicosatrienoic acids⁷⁰. By contrast, only plant-derived cannabinoids (i.e. ⁹Δ-THC, cannabinol and cannabidiol) activate TRPV2 receptors, although with low affinity (> 10μM)⁷¹. The low affinity of AEA at TRPV1 receptors has raised questions whether this lipid might serve as an actual endovanilloid ligand^{72,73}. Nevertheless, in vitro studies indicate that inhibition of FAAH activity, which results in AEA elevation in the body (see below), enhances AEA potency at TRPV1⁷⁴.

The existence of a crosstalk between CB₁ and TRPV1 is evidenced by studies showing that CB₁ stimulation can alter the functional state of TRPV1 by modulating its phosphorylation state^{75,76}.

Cannabinoids that selectively activate TRPV1 (like arachidonoyl-chloro-ethanolamide, ACEA) or TRPA1 receptors (like WIN55212-2) produce homologous and cross-desensitization via Ca²⁺-dependent^{76,77} and -independent mechanisms (for review see [78]). This phenomenon, however, has not been investigated in the CNS.

PPAR receptors—Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that heterodimerize with the retinoid X receptor and bind to specific DNA sequences, termed PPAR response elements, to enhance the expression of target genes. There are three isoforms: α, β/δ and γ. All subtypes are expressed in neuronal and glial cells of the peripheral and CNS^{79,80}. PPAR have been involved in the regulation of fatty acid and lipoprotein metabolism⁸¹, energy homeostasis⁸², inflammation⁸³, cell proliferation and apoptosis⁸⁴, insulin sensitivity and food intake⁸⁵. Also, PPARγ agonists, such as

thiazolidinediones, have anti-inflammatory and anti-oxidant properties in animal models of CNS injury, Parkinson's disease, cerebral ischemia and multiple sclerosis⁸⁶⁻⁸⁸.

Unsaturated fatty acids and numerous eicosanoids are thought to be the endogenous ligands of PPAR, with EC₅₀ values ranging from 0.5 to 20 μ M⁸⁹. Because of their large ligand-binding domain, however, PPAR are relatively promiscuous and can be activated by different compounds, including cannabinoids⁹⁰. For example, WIN55212-2 and the endocannabinoids AEA, noladin ether and virodhamine bind to the purified PPAR α binding domain and increase PPAR α transcriptional activity⁹¹. AEA can also bind to PPAR γ to promote adipocyte differentiation⁹², a well-known property of PPAR γ agonists. The functional role of endocannabinoid-mediated activation of PPAR in the CNS remains elusive. Recently, Melis et al. (2008) have shown that FAAH inhibition suppresses nicotine-induced excitation of dopamine neurons in rat ventral tegmental area (VTA) via activation of brain PPAR α . This effect is mimicked by the AEA structural analogs oleylethanolamide (OEA) and palmitoylethanolamide (PEA), is non-genomic and possibly dependent on the activation of a non-yet identified tyrosine kinase⁹³.

1.2 Endocannabinoids and metabolizing enzymes

The endocannabinoids are a family of endogenous signaling lipids that activate cannabinoid and non-CB₁/CB₂ receptors (Fig. 1). Unlike classic neurotransmitters, endocannabinoids are not stored in synaptic vesicles, but are produced on demand by stimulus-dependent cleavage of membrane phospholipid precursors⁹⁴⁻⁹⁵. To date, arachidonylethanolamide (anandamide, AEA)⁹⁴⁻⁹⁶ and 2-arachidonylglycerol (2-AG)⁹⁷⁻⁹⁸ are the best-characterized examples.

AEA acts as partial agonist at CB₁ and CB₂ receptors and mimics most of the pharmacological effects produced by cannabinoid drugs *in vitro* (e.g. inhibition of adenylyl cyclase activity) and *in vivo* (e.g., analgesia, hypomotility, hypothermia⁹⁶⁻⁹⁹). AEA is synthesized from the deacylation of the phospholipid precursor N-arachidonoyl phosphatidylethanolamine (NAPE) via a Ca²⁺⁺-dependent process involving a NAPE-specific PLD activity¹⁰⁰. In rat hippocampus, NAPE-PLD is located presynaptically on excitatory terminals, in the proximity of intracellular calcium stores¹⁰¹. The location of the biosynthetic machinery of AEA is remarkably different from that of 2-AG (post-synaptic), suggesting distinct physiological roles for these two endocannabinoids. Interestingly, no significant changes in the levels of brain AEA have been found in NAPE-PLD knockout mice¹⁰², indicating the existence of additional biosynthetic pathways, such as those initiated by the recently identified $\alpha\beta$ -hydrolase 4 (Abhd4)¹⁰³ or by the sequential action of PLC and phosphatases¹⁰⁴.

The biological actions of AEA are terminated via a two-step process consisting of facilitated diffusion into cells via a carrier-mediated transport¹⁰⁵⁻¹⁰⁷, followed by enzymatic hydrolysis via a fatty acid amide hydrolase (FAAH)^{108, 109} (Fig. 1). Skepticism has been raised about the existence of an endocannabinoid transporter^{110,111}, whose molecular identity remains unknown. Alternative mechanisms have been described for the uptake of endocannabinoids, including intracellular sequestration via unidentified lipid-binding protein(s)¹¹¹; passive diffusion driven by FAAH^{110,112,113}; and caveolae-mediated endocytosis¹¹⁴.

To date, two FAAH isoforms with approximately 20% sequence homology have been identified, FAAH-1¹⁰⁹ and FAAH-2¹¹⁵. The latter, is expressed in higher vertebrates including primates, but not in rodents.

In FAAH-1 knockout mice, as well as after pharmacological inhibition of FAAH in wildtypes, AEA (and in some tissues 2-AG) is elevated throughout the body and the CNS^{113,116}. Recent report shows that blockade of FAAH by URB597 reduces brain 2-AG levels in non-human primates¹¹⁷ and rat striatal slices via a mechanism involving activation of TRPV1 receptors

and inhibition of DAGL118. The decrease of 2-AG in rat brain, however, has not been confirmed by other groups in vivo, even at doses of URB597 that completely inhibit FAAH activity^{66,116,119,120}, and may be restricted to specific brain areas and/or be dependent on the physiological status of the region examined.

Enzymes other than FAAH can metabolize AEA. For example, AEA is a substrate for cyclooxygenase-2 (COX-2)¹²¹ (Fig. 1). The result of this cyclooxygenation is the formation of prostaglandin-like compounds (prostaglandins)¹²², which have weak effects at prostaglandin receptors. Administration of exogenous AEA to FAAH^{-/-} mice leads to the production of detectable levels of prostaglandins¹²³, suggesting that the COX-2 metabolic pathway becomes physiologically relevant under conditions affecting FAAH activity or promoting COX-2 upregulation, as in the case of neurodegenerative diseases¹²⁴ or tissue damage¹²⁵. In addition, lipoxygenases (12-LOX and 15-LOX)^{126,127} and P450 (Fig.1), including its major brain isoform CYP2D6¹²⁸, convert AEA into biologically active metabolites that exert their biological actions via cannabinoid, PPAR α and TRPV1 receptors^{129,130}. However, the functional role of these substances in the CNS has not been determined.

In addition to its ability to act as an endovanilloid at TRPV1¹³¹ and bind to GPR55 receptors⁵² (see above), AEA can activate PPAR α and γ subtypes^{91,92,132} (Fig. 1). The majority of these experiments, however, have been carried out in cells transiently transfected with different PPAR subtypes and have not been unambiguously confirmed in neurons. In rats, electrophysiology studies show that endocannabinoid elevation via FAAH inhibition suppresses nicotine-induced activation of VTA dopamine neurons via PPAR α ⁹³. This effect is not mimicked by intracerebral administration of the stable AEA analog, metanandamide (mAEA), suggesting that other acylethanolamides that serve as substrates for FAAH, i.e. PEA or OEA, might be involved in this response. In agreement with this hypothesis, the analgesic properties of mAEA are preserved in PPAR α knockout mice, whereas those of OEA and PEA are not¹³³.

2-AG is a full agonist at cannabinoid receptors¹³⁴, although less potent than AEA³, stimulates GPR55 receptors in transfected cells⁵¹, acts as a retrograde messenger on pre-synaptic CB₁ receptors located on excitatory and inhibitory synapses to inhibit neurotransmitter release^{31,135,136}, and is an autocrine mediator of post-synaptic slow self-inhibition (SSI) of neocortical interneurons¹³⁷. In the brain, 2-AG is synthesized via a receptor/Gq-initiated sequential hydrolysis of inositol phospholipids containing arachidonic acid by PLC β and diacylglycerol lipase (DAGL)^{98,138-140}. In 2003, Bisogno et al. showed that the expression of the two DAGL isoforms, α and β , switch from neuronal axons to post-synaptic dendritic fields during brain development, thus providing a spatial and temporal regulation of 2-AG signaling that is consistent with its functional role in the embryonic (promotion of axonal growth) as compared to the adult brain (retrograde signaling)¹⁴⁰. Recent anatomical studies have confirmed that DAGL α is the main isoform in adult hippocampal and cerebellar post-synaptic neurons facing CB₁-expressing terminals¹⁴¹⁻¹⁴³. Studies carried out in PLC β -disrupted mice indicate that activation of PLC β 1 via M1/M3 muscarinic receptors and stimulation of PLC β 4 by 1-mGluR trigger the release of 2-AG in the hippocampus and cerebellum, respectively^{144,145}. Alternatively, 2-AG might be formed via the combined action of PLA₁ and lyso PI-PLC^{98,146}.

Experimental evidence suggests that 2-AG is accumulated intracellularly by the same carrier protein mediating AEA re-uptake^{147,148} and that this putative carrier is not regulated by 2-AG metabolizing enzymes, at least in vitro¹⁴⁹.

Although 2-AG can be metabolized by FAAH¹⁵⁰, the serine hydrolase MAGL is the major hydrolyzing enzyme in intact neurons^{151,152}. Pharmacological blockade of MAGL elevates 2-

AG in the dorsal midbrain without affecting AEA levels¹⁵³ and potentiates 2-AG effects in vitro and in vivo¹⁵⁴⁻¹⁵⁶. The presynaptic co-localization of MAGL and CB₁ receptors in several brain regions further confirms the role of this enzyme in terminating 2-AG-mediated retrograde signaling^{157, 158}.

As in the case of AEA, other enzymes, including COX-2 and LOX, can metabolize 2-AG¹⁵⁹. The COX-2-derived metabolite, PGE₂ glycerol ester has been shown to modulate miniature inhibitory currents and to affect long-term potentiation (LTP) in mouse hippocampus in a CB₁-independent fashion¹⁶⁰. Interestingly, depolarization-induced suppression of inhibition (DSI) in rat hippocampus, a phenomenon modulated by 2-AG, is enhanced by COX inhibitors (such as meloxicam and nimesulide), suggesting a role of 2-AG metabolites in synaptic plasticity¹⁶¹. On the other hand, the LOX-derived 2-AG metabolite, 15-hydroxyeicosatetraenoic acid glyceryl ester (15-HETE-G), increases PPAR α transcriptional activity¹²⁷. Recently, a novel MAGL activity localized in mitochondrial and nuclear fractions has been shown to metabolize 2-AG in microglia cells¹⁵⁰.

Unlike AEA, 2-AG does not directly activate TRPV1 or PPAR receptors but can bind to non-CB₁/CB₂ targets as suggested by the observation that the behavioral effects induced by N-arachidonyl maleimide (NAM), a selective MAGL inhibitor with in vivo efficacy, are only partially reversed by the CB₁ antagonist rimonabant¹⁵⁵.

In summary, the endocannabinoids are emerging as important signaling lipids in the CNS that modulate excitatory and inhibitory neurotransmission and synaptic plasticity. These lipids do not bind metabotropic cannabinoid receptors exclusively, but display significant promiscuity as they activate ionotropic and nuclear targets, i.e. TRPV1 and PPAR receptors, as well as other non-CB₁/CB₂ receptors. The characterization of the endocannabinoid biosynthetic and metabolic pathways has led to the discovery of novel molecular entities, such as NAPE-PLD, FAAH and MAGL, which can be targeted pharmacologically not only to manipulate endocannabinoid levels, but also to investigate the role of the endocannabinoid system in physiological and pathological conditions. In this regard, the development of more selective FAAH/MAGL inhibitors with significant in vivo activity is crucial to understand the specific biological actions of AEA, 2-AG and their metabolites in living animals.

2. In vivo pharmacology of endocannabinoids

Given the wide distribution of cannabinoid receptors in brain, it is not surprising that endocannabinoids have been implicated in numerous CNS functions including appetite regulation¹⁶², mood control^{1,163,164}, reward^{165,166}, motor function¹⁶⁷, immune response^{168, 169} and pain^{170,171}. Since these properties are already covered by existing reviews they will not be discussed here. This section provides an overview of the behavioral pharmacology of AEA and 2-AG, including examples of apparent differences between endocannabinoids and prototype cannabinoids and behavioral effects that are apparently not mediated by CB₁ receptors. This review focuses primarily on the mouse “tetrad” (i.e., hypothermia, catalepsy, hypolocomotion, and antinociception)¹⁷² and drug discrimination assays¹⁷³. The tetrad represents a relatively selective procedure to detect cannabinoid agonism and has been widely used to provide systematic and comprehensive pharmacological profiles for a large number of cannabinoid drugs¹⁷²⁻¹⁷⁴.

Drug discrimination is a sensitive behavioral assay used to classify drugs according to a shared pharmacological mechanism¹⁷³. In this procedure, animals are trained to make an operant response (e.g., lever pressing) to obtain a reinforcer (e.g., food) after receiving a training Δ^9 -THC, and a different drug (e.g., operant response after receiving vehicle). Once the animal discriminates between the training drug and vehicle, it is tested with different doses of another drug that is either pharmacologically related (e.g., cannabinoid agonist) or not. During testing,

the animal selects the operant associated with either the training drug or vehicle to obtain a reinforcer. Typically, only drugs sharing a common pharmacological mechanism with the training drug will produce responses on the corresponding lever. The test drug can also modify the effect of the training drug to produce such a response. By contrast, pharmacologically unrelated drugs typically will not modify the effects of the training drug.

2.1 Behavioral pharmacology of AEA

AEA can readily enter the brain following intravenous administration and is rapidly metabolized by FAAH: brain levels are elevated within 5 min and return to baseline in 15 min¹⁷⁵. Nevertheless, AEA-induced behaviors last longer than 15 min. For example, systemic administration of AEA produces effects in the tetrad for 30-60 min¹⁷⁶. The discrepancy between AEA brain levels and the manifestation of its behavioral actions has been attributed to AEA conversion into biologically active metabolites, i.e. arachidonic acid, which produce similar effects^{177, 178}.

CB₁ receptors have long been thought to mediate the behavioral effects of cannabinoids. However, studies using pharmacological or gene knockout approaches to disrupt CB₁ receptor function indicate that targets other than CB₁ receptors are involved in some of the in vivo effects of AEA. Although, both Δ^9 -THC and AEA are qualitatively similar in the mouse tetrad¹⁷⁶, the CB₁ antagonist rimonabant fully antagonizes only Δ^9 -THC¹⁷⁹ and shows less potency at reversing the antinociception induced by intrathecal AEA as compared to Δ^9 -THC¹⁸⁰. In addition, AEA produces catalepsy and antinociception in both CB₁ knockout mice and wildtype controls, Δ^9 -THC on these measures is markedly attenuated in CB₁ knockout mice only¹⁸¹. On the other hand, the suppressive effect on locomotion produced by AEA Δ^9 -THC and is similar in CB₁ knockouts and wild-type littermates, suggesting that, in mice, both compounds act at non-CB₁ receptors to decrease locomotor activity¹⁸¹ (see also [182]). Noteworthy, the role of CB₁ receptors in cannabinoid-induced locomotor responses might vary among species. In rats, for example, CB₁ antagonists can reverse Δ^9 -THC-induced reverse effects on horizontal motor activity¹⁸³.

Drug discrimination studies further support the hypothesis that CB₁ receptors do not mediate all the behavioral effects of exogenously administered AEA. In rats discriminating Δ^9 -THC, AEA did not share discriminative stimulus effect with Δ^9 -THC, or produced partial Δ^9 -THC-like effects even at doses that decreased the overall rate of lever pressing¹⁸⁴⁻¹⁸⁶. These results were confirmed by a drug discrimination study conducted in our laboratory. In this study, C57BL/6J mice were trained to discriminate Δ^9 -THC (3.2 mg/kg i.p.) from vehicle using an operant nose-poking procedure described elsewhere¹⁸⁷. As shown in Figure 2, Δ^9 -THC produced dose-dependent increases in Δ^9 -THC operant behavior. The cannabinoid agonists CP55940 and WIN55212-2 mimicked Δ^9 -THC dose-dependently, whereas AEA did not when studied up to a dose (56 mg/kg) that markedly decreased response rate. These results, in conjunction with previous data showing that the CB₁ antagonist rimonabant antagonizes the discriminative stimulus effects of Δ^9 -THC, CP55940 and WIN 55212-2¹⁸⁷, suggest that AEA produces behavioral effects through non-CB₁ receptors in mice.

Different results have been obtained Δ^9 -THC, where in AEA did or did not share discriminative Δ^9 -THC depending on the doses chosen and/or the route of drug administration (intravenous versus systemic¹⁸⁸, respectively). Interestingly, rimonabant antagonized AEA, Δ^9 -THC and a variety of other cannabinoid agonists with similar potency¹⁹⁴, indicating that in non-human primates AEA pharmacology is CB₁-dependent and strikingly similar to that of prototype cannabinoid agonists. The divergent pharmacological profiles of AEA in rodents versus primates may depend on differences in metabolism and/or in the contribution of AEA metabolites to behavioral effects. Other factors, such as species-specific differences in receptor type (i.e., CB₁ and TRPV1) distribution and expression might be also important.

The contribution of non-CB₁ receptor sites to the in vivo effects of AEA in the CNS has not been extensively studied. In a recent report⁶⁵, AEA produced dose-dependent effects in a five-choice serial reaction-time task indicative of disruption of attention; these effects were attenuated by the TRPV1 antagonist capsazepine but not by rimonabant or the PPAR antagonist MK886. However, the extent to which these results reflect actions of AEA versus its metabolites is not clear. AEA also suppressed nicotine-induced excitation of dopamine cells in the VTA via a PPAR α -mediated mechanism⁹³.

2.2 Behavioral pharmacology of AEA analogs and FAAH inhibitors

The differences in AEA Δ^9 -THC and pharmacological profiles observed in the drug discrimination and tetrad procedures may be attributed to: (1) direct activity of AEA at non-CB₁ receptors. Indeed, AEA differed from Δ^9 -THC even when studied 5 min from after administration, when its brain levels were presumably elevated^{179,181,185}. (2) Rapid formation (i.e., within 5 min) of one or more AEA metabolites acting via non-CB₁ receptors.

Two strategies to limit AEA metabolism will be reviewed here: 1) administration of metabolically stable AEA analogs; and 2) pharmacologic inhibition or genetic deletion of FAAH.

Metabolically stable AEA analogs—Among these compounds, (R)-methanandamide (mAEA) has been the most extensively characterized¹⁸⁹. While AEA binding affinity for CB₁ is significantly increased (approximately 15-fold) in mice lacking the FAAH gene, mAEA affinity for this receptor remains unchanged¹⁷⁷, suggesting that mAEA is not a FAAH substrate. Unlike AEA, mAEA shared discriminative stimulus effects with Δ^9 -THC¹⁸⁵, which were antagonized with similar potency by rimonabant^{185,190}. Another stable AEA analog, AM1346¹⁹¹, shared discriminative stimulus effects with both Δ^9 -THC and mAEA in separate groups of rats trained to discriminate each respective drug¹⁹⁰; also, in this case rimonabant produced surmountable antagonism of AM1346 under all the conditions studied. Together, these results indicate that, in rats, AEA analogs have a behavioral profile that appears to be more CB₁-dependent than that of AEA. These analogs, however, produce behavioral effects that are not always consistent. For example, in rats Δ^9 -THC and AM1346 produced circling behavior¹⁹¹, whereas mAEA did not¹⁹². The behavioral profile of AEA analogs (mAEA, ACPA) in non-human primates is also CB₁-dependent. Indeed, in rhesus monkeys, both mAEA and arachidonylcyclopropylamide (ACPA), another AEA analog resistant to FAAH inhibition¹⁹³, shared discriminative stimulus effects with Δ^9 -THC through a common CB₁-dependent mechanism¹⁹⁴.

By contrast, studies in mice failed to show that AEA analogs act via CB₁ receptors. As shown in Fig. 2, mAEA and ACPA did not share discriminative stimulus effects with Δ^9 -THC up to doses that decreased the overall rate of responding (bottom right panel). Other studies carried out in our lab also show that in mice all AEA analogs and prototype cannabinoid agonists produce hypothermia (Fig. 3); the effect of the former, however, was not CB₁-dependent, whereas rimonabant antagonized the hypothermic effects of Δ^9 -THC, WIN55212-2 and CP55940 (Fig. 3). Also, when measuring decreases in operant responding for food¹⁹⁵, rimonabant antagonized Δ^9 -THC antagonized but not mAEA, and the effects of mAEA were not modified in CB₁ knock out mice unlike those of Δ^9 -THC. These results are perplexing in light of ex vivo studies showing that mAEA binds to CB₁ receptors in mouse brain after systemic administration at the same doses chosen in the studies reported above¹⁹⁶. Therefore, it is plausible to hypothesize that AEA analogs could bind to non-CB₁ receptors and produce behavioral effects that interfere with their putative CB₁-dependent actions.

Pharmacological inhibition and genetic ablation of FAAH—The non-selective FAAH inhibitor PMSF has been shown to enhance AEA-induced immobility, antinociception, and hypoactivity in mice via CB₁ receptors¹⁹⁷. At relatively large doses, PMSF did produce cannabinoid-like effects in the tetrad, leaving open the possibility that additive effects of PMSF and AEA contributed to the leftward shifts of the AEA dose-response curve reported by Compton and Martin (1997). Nevertheless, since PMSF can act at other esterases and, for example, inhibit the metabolism of acetylcholine by blocking acetylcholinesterase¹⁹⁸, we cannot rule out that the interaction with this and/or other neurotransmitters might contribute to its ability to enhance AEA potency in vivo.

Recently, significant progress has been made in developing FAAH inhibitors with suitable bioavailability and selectivity for in vivo studies. Among these, URB597^{116, 199} and OL135^{200,201}, have been extensively studied. In rodents, both compounds elevated brain AEA levels^{116,199} and increased AEA-induced antinociception, immobility and hypothermia dose-dependently, whereas they had no effect when administered alone^{200,202}. In rats, URB597 did not share discriminative stimulus effects with Δ^9 -THC¹¹⁹; however, its combination with AEA did, in a CB₁-dependent fashion¹⁸⁶.

The in vivo effects of URB597 and AEA have been further examined in other behavioral assays, such as place conditioning²⁰³. When these compounds were administered separately, no place conditioning occurred. When given together, however, rats did not prefer the environment associated with URB597+AEA (i.e. place aversion), suggesting an aversive effect similar to that obtained with other CB₁ agonists²⁰³. Whether this result is a consequence of URB597-induced AEA elevation is not clear, as AEA per se did not produce aversion when given at the doses studied (see also [204]). In contrast to the results obtained with AEA alone, the effects of URB597+AEA were attenuated by CB₁ antagonists in all the behavioral paradigms reported above^{186,203}. Collectively, these data provide evidence that URB597 increases AEA potency to produce CB₁-dependent effects in vivo.

Interestingly, systemic administration of URB597 alone also produces some behavioral effects of therapeutic value including anxiolytic-like and anti-depressant activity via a CB₁-mediated mechanism^{116,119}.

In FAAH knockout mice, AEA produced dose- and CB₁-dependent effects in the tetrad^{113, 202,205,206}. Interestingly, AEA-induced hypothermia, antinociception and catalepsy were significantly reduced in double knockout mice lacking FAAH and CB₁ receptors as compared to mice lacking only FAAH²⁰⁶, suggesting that AEA metabolites may contribute to the divergent pharmacological profiles of AEA in intact and FAAH knockout mice.

2.3 Behavioral pharmacology of 2-AG and MAGL inhibitors

2-AG is more rapidly metabolized than AEA (i.e. 2-fold)²⁰⁷ and, when administered exogenously, it presents a behavioral profile that is not entirely consistent with CB₁ receptor agonism. For example, while Δ^9 -THC, CP 55940 and WIN 55212-2 while attenuate CB₁ antagonist-induced vomiting in shrews²⁰⁸, 2-AG is ineffective or rather induces vomiting on its own. In addition, the 2-AG emetic effects are attenuated by both Δ^9 -THC and non-emetic doses of rimonabant²⁰⁹. Although these data might indicate that 2-AG exhibits some cannabinoid antagonist actions, blockade of the anti-emetic effects of Δ^9 -THC by non-emetic doses of 2-AG has not been demonstrated. Alternatively, a 2-AG metabolite (i.e. arachidonic acid) may mediate 2-AG biological properties via a non-CB₁ receptor mechanism. In agreement with this possibility, in mice the effects of 2-AG on hypoactivity, antinociception, and hypothermia were not attenuated by rimonabant but they were mimicked by arachidonic acid¹⁷⁷.

Selective MAGL inhibitors suitable for *in vivo* investigations have been identified very recently. URB602, which is slightly more selective for MAGL than FAAH, has been shown to mimic URB597 in reversing stress-induced analgesia when injected directly into the periaqueductal gray region¹⁵³. Another non-selective MAGL inhibitor, JZL184, produced antinociception, hypothermia and hypolocomotion in mice at doses blocking approximately 90% and 60% of MAGL and FAAH activity, respectively¹⁵⁶. However, since high doses of URB602 and JZL184 can elevate AEA by blocking FAAH activity, it is still not clear whether the *in vivo* effects of either drugs can be attributed to 2-AG only. In 2008, Burston et al. showed that N-arachidonoyl maleimide (NAM) increases 2-AG, but not AEA, via selective inhibition of MAGL and potentiates 2-AG-induced stimulation of CB₁ receptors *in vitro*¹⁵⁵. Moreover, the co-administration of ineffective doses of NAM and 2-AG elicited 2-AG dose-dependent behavioral responses in the mouse tetrad¹⁵⁵.

Collectively, the studies reviewed above show that exogenously administered AEA and 2-AG produce robust behavioral effects that can be strikingly different from those obtained following administration of plant-derived and synthetic cannabinoids, likely due to rapid formation of behaviorally active metabolites. Additional studies are needed to define the contribution of these metabolites to the behavioral effects of endocannabinoids. These studies will require effective strategies to prevent the formation of metabolites (i.e., via selective blockade of FAAH and MAGL), as well as systematic evaluation of the behavioral effects of the metabolites alone and in combination with their parent ligands. Moreover, while CB₁ receptors are clearly important for the behavioral effects of endocannabinoids, the role of other non-CB₁/CB₂ receptors *in vivo* requires further investigations.

3. Therapeutic implications

Given the short half-life of endocannabinoid compounds, which limit their use *in vivo*, there has been considerable interest in exploring possible therapeutic applications of FAAH and MAGL inhibitors in pre-clinical settings. In this regard, most studies have been focusing on FAAH inhibition due to the development of selective and bio-available inhibitors (URB597), as well as genetically modified mice lacking the FAAH gene.

This section reviews the role of endocannabinoids and URB597 in conditions characterized by changes and/or dysfunction of DA transmission, such as drug abuse and Parkinson's disease (PD), and summarizes recent studies addressing the abuse liability of FAAH inhibitors. The effects of these drugs on neuropathic and inflammatory pain, anxiety, depression and other psychiatric and neurological disorders have been reviewed elsewhere^{164,210-212}.

3.1 Cannabinoid-dopamine crosstalk and Parkinson's disease

Several lines of evidence indicate the existence of a cross talk between the endocannabinoid and dopaminergic systems in brain areas regulating motor function such as the basal ganglia: 1) For example, CB₁ and D₁/D₂-like receptors are co-localized in striatal neurons^{213,214}; 2) endocannabinoids and FAAH inhibitors influence the firing activity of dopaminergic neurons^{63,93}, as well as dopamine release *in vivo*²¹⁵⁻²¹⁷; and 3) stimulation of dopamine receptors increases AEA levels in the basal ganglia^{218,219}. The functional significance of dopamine-induced AEA increase is not completely understood. Behavioral studies suggest that it may serve as an inhibitory feedback signal countering dopamine-induced motor activity^{218,220,221}. Abnormalities in DA signaling - as reported in PD and related animal models - may disrupt this feedback mechanism²¹⁹ and lead to a functional state characterized by motor disturbances.

Increased CB₁ receptor binding and CB₁ mRNA levels have been reported in the brain of MPTP-treated primates²²² and in rats with 6-OHDA-lesions²²³. In addition, the 6-OHDA-

lesion has been shown to decrease AEA levels in the ipsilateral caudate-putamen²¹⁹. Although other groups have reported elevated endocannabinoid levels in rat striatum following dopamine depletion^{224,225}, there is no electrophysiological evidence supporting increased endocannabinoid tone in 6-OHDA-treated rats²²⁶. Interestingly, administration of levodopa, the mainstay treatment for PD, does not elevate AEA in the basal ganglia of 6-OHDA-treated rats²¹⁹ while it causes upregulation of striatal CB₁ receptors²²⁷. Collectively, these observations indicate that degeneration of nigro-striatal projections dramatically affects endocannabinoid transmission, and administration of levodopa does not correct these abnormalities.

Studies on the potential therapeutic utility of cannabinoid agonists and antagonists in PD have produced conflicting results, particularly when comparing rodent and primate models²²⁸⁻²³². On the other hand, experimental evidence suggests that a deficiency in endocannabinoid transmission may contribute to the development of levodopa-induced dyskinesias (LID), a disabling motor complication resulting from long-term use of levodopa²³³. In keeping with this hypothesis, several reports have shown that LID are alleviated by activation of CB₁ receptors^{66,219,234}. Cannabinoid agonists may exert anti-dyskinetic effects by modulating the glutamatergic input from cortico-striatal afferents^{225,235} and/or by “rescuing” endocannabinoid-mediated synaptic plasticity in the striatum²²⁶, which are both dysfunctional in 6-OHDA-treated rats^{226,236}. Although a double-blind, placebo-controlled trial failed to recognize any effect of cannabis preparations on LID²³⁷, this assessment was based on diary data from patients, which are often limited by inaccurate labeling of dyskinetic symptoms²³⁸.

FAAH inhibitors, which are known to reduce dopamine-induced hyperactivity in mice²³⁹ and hyperkinesia in a rat model of Huntington’s disease²⁴⁰, failed to produce an anti-dyskinetic effect when administered alone in rats⁶⁶, suggesting that AEA elevation is not sufficient to attenuate LID. The lack of effect of URB597 as compared to cannabinoid agonists like WIN55212-2 may depend on the different pharmacological profiles of these compounds (see above) and on the ability of AEA to activate TRPV1 receptors¹³¹, particularly under conditions in which FAAH activity is inhibited⁷⁴. In support of this hypothesis, systemic administration of URB597 in combination with the TRPV1 antagonist capsazepine produced a significant anti-dyskinetic effect, suggesting that the beneficial actions of CB₁ stimulation on LID may be counteracted by TRPV1 agonism. These findings differ from those reported by Lee et al. (2006), showing that URB597 alone or stimulation of TRPV1 receptors by capsaicin can attenuate levodopa-induced hyperactivity in reserpine-treated rats²⁴¹. These discrepancies may be due to the different animal models and/or the type of behavioral measure used (vertical motor activity), which models stereotypies rather than dyskinesias²⁴². Whether the anti-dyskinetic effects of URB597+capsazepine are mediated via activation of CB₁ receptors remains unclear as the CB₁ antagonist AM251 attenuated only URB597+capsazepine-induced suppression of oro-facial dyskinesias, whereas it had no effect on other LID subtypes⁶⁶. Thus, targets other than CB₁ receptors are likely to play a role in axial and limb dyskinesias.

3.3 Pre-clinical assessment of drug abuse liability

The use of cannabinoid receptor agonists as therapeutics has been limited by concerns over their direct actions at CB₁ receptors, which mediate the adverse side effects and abuse liability of marijuana. Pre-clinical studies show that FAAH inhibitors have anxiolytic and analgesic activity^{116-119,203}. In general, pharmacotherapies that produce similar effects (such as benzodiazepines and μ opioid agonists) are associated with significant abuse and dependence liability. By contrast, other cannabinoid ligands, such as the CB₁ antagonist rimonabant, which

has until recently been prescribed for obesity, have not exerted actions indicative of abuse potential²⁴³.

Drug self-administration is the most commonly used pre-clinical screen of abuse liability. In this procedure, an animal makes an operant response (i.e. lever press) to receive a dose of drug, and drug-maintained operant responding: (1) provides an index of abuse liability, and (2) can be used to evaluate pharmacotherapies that modify this behavior²⁴⁴. Similarly, modification of an animal preference for an environment previously paired with an abused drug (*drug place preference test*²⁴⁵) can be used to assess the ability of a new compound to reverse addictive behaviors. Since most drugs decrease self-administration when administered at large doses, modifications of drug self-administration and place preference are best interpreted when compared to a non-drug reinforcer, such as food. Thus, a test compound that selectively decreases drug-(but not food-) maintained responding is considered a better pharmacotherapy for drug abuse than a compound decreasing responding for food and drug equally.

When operant responding is no longer reinforced with drug delivery, it significantly declines (extinction). Thereafter, *reinstatement* of operant responding can be obtained by non-contingent administration (i.e. not resulting from lever pressing) of the drug that was previously self-administered, or by another drug, or by a stimulus previously paired with contingent drug delivery²⁴⁶. Reinstatement is used to index *relapse*, defined as re-initiation of drug use after a period of abstinence, although reinstatement and relapse are not entirely concordant²⁴⁷.

Despite the widespread recreational use of cannabinoids in the form of marijuana smoking, Δ^9 -THC has not exerted addictive properties in numerous pre-clinical studies (for review see 248). For example, non-human primates and rats do not reliably self-administer Δ^9 -THC and Δ^9 -THC does not produce place preference, but rather place aversion²⁴⁹. However, when the Δ^9 -THC was adjusted dose to be comparable of to that inhaled from a marijuana cigarette, robust CB₁-dependent self-administration was observed in squirrel monkeys^{117,250,251}. Similarly, AEA and mAEA, but not URB597, were robustly self-administered in a CB₁-dependent fashion^{117,252}.

The lack of URB597 self-administration was consistent with previous observations showing that this drug does not share discriminative stimulus effects with Δ^9 -THC^{119, 186} and does not produce place conditioning (preference or aversion)²⁰³ or enhance electrical brain stimulation²⁵³ in rats. Collectively, these studies indicate that URB597 has no abuse liability. It is unknown, however, whether these observations can be extended to other FAAH inhibitors.

Modification of the abuse liability of other drugs—The combined use of marijuana and other abused drugs (e.g., ethanol and μ opioid agonists, such as heroin) appears to be mediated, in part, by pharmacologic (receptor-mediated) interactions, and there is concern that cannabinoids can enhance the abuse liability of other drugs. For example, in rats, direct-acting CB₁ agonists increase ethanol consumption²⁵⁴⁻²⁵⁶ and heroin self-administration²⁵⁷. By contrast, URB597 does not share these effects^{257,258} nor does it enhance Δ^9 -THC or cocaine self-administration in squirrel monkeys¹¹⁷. In addition, URB 597 attenuates ethanol withdrawal, as evidenced by decreased anxiety-like effects emerging in rats upon discontinuation of chronic ethanol treatment (Cippitelli et al. 2008). However, different laboratories have shown that FAAH knockout mice exhibit increased preference for ethanol relative to water²⁵⁹⁻²⁶¹. Moreover, URB597 increased ethanol preference in normal but not in FAAH or CB₁ knockout mice²⁶⁰, suggesting that URB597-induced effects on ethanol preference depend on the presence of both FAAH (i.e., increased AEA levels) and CB₁ receptors²⁶¹. On the other hand, place conditioning to ethanol did not differ between FAAH knockout and wildtype mice²⁶⁰.

Pre-clinical studies examining interactions between FAAH inhibition and nicotine abuse have yielded different results. In rats, URB597 reduced nicotine place preference and attenuated acquisition of lever pressing for nicotine, as well as the reinstatement of this behavior²⁶². These effects might be due to URB597 attenuating nicotine-induced elevation of dopamine levels in the nucleus accumbens⁹³, which has been associated with nicotine intake²⁶³. Other studies, however, indicate that in mice low doses of URB597 or deletion of the FAAH gene increase nicotine potency to produce place preference, whereas URB597 has no effect on nicotine-induced hypothermia or antinociception²⁶⁴. In addition, nicotine withdrawal (indexed by observable signs and conditioned place aversion) was enhanced not only in FAAH knockout mice but also by URB597 in wildtype littermates²⁶⁴. Together, these data suggest that URB597 does not increase the abuse liability Δ^9 -THC of (marijuana) opioid and may even attenuate some aspects of ethanol withdrawal in animals; however, the effects of URB597 on nicotine and alcohol abuse remain unclear and additional pre- and clinical studies are necessary.

CONCLUSIONS

The pharmacological modulation of the endocannabinoid system has shown therapeutic potential for the treatment of numerous CNS pathologies. In particular, the interaction between endocannabinoids and the dopamine system may lead to the development of cannabinoid-based pharmacological strategies for the treatment of disorders characterized by dopamine dysfunction, including drug abuse and PD. For example, there is pre-clinical evidence that some cannabinoid agonists protect nigrostriatal dopaminergic neurons in different models of PD²⁶⁵⁻²⁶⁷. However, clinical investigations are necessary to test the neuroprotective properties of these compounds in PD patients. Furthermore, it is unknown whether endocannabinoids-enhancing drugs share similar neuroprotective properties in pre-clinical and clinical settings.

Limited studies are available on the effects of cannabinoids on the motor complications caused by long-term use of anti-parkinsonian medications. So far, clinical investigations have been inconclusive whether cannabinoid agonists, such as THC, might relieve dyskinetic symptoms in PD. In addition, the antidyskinetic properties of endocannabinoids-enhancing drugs have not been tested in the clinic, despite promising results obtained in animal models. Experimental evidence indicates that CB₁ receptors are not the only anti-dyskinetic targets activated by the endocannabinoids, and that TRPV1 receptors might be critical in modulating the response of endocannabinoids-enhancing drugs. Thus, studies aimed at investigating the molecular mechanisms underlying the CB₁/TRPV1 crosstalk may lead to important advancements in the pharmacotherapy of dyskinesias.

Drugs that regulate endocannabinoids levels by inhibiting their metabolism (FAAH and MAGL inhibitors) are particularly attractive therapeutically as they appear to have low or no abuse liability. They may also produce less undesired side effects as they can locally target sites in the brain where endocannabinoids are produced on demand, thus avoiding the widespread activation of CB₁ receptors obtained with cannabinoid agonists. Behavioral studies support the notion that endocannabinoids and their metabolic inhibitors have different pharmacologic profiles than prototype cannabinoids. These divergences may be ascribed to differences in the pharmacokinetics of endocannabinoids, which also may vary across the animal species used in behavioral studies, as well as to non-CB₁/CB₂ targets activated by these compounds. However, the physiological roles played by these receptors have not been fully explored and may imply new beneficial actions for endocannabinoid-based therapies.

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Figure 1. Receptor targets and metabolic enzymes of anandamide

Anandamide can activate GPCR receptors, such as CB₁, CB₂ and GPR55, ionotropic (TRP) receptors, such TRPV1 and the nuclear receptors PPARs (alpha and gamma subtypes). Anandamide is hydrolyzed into ethanolamine and arachidonic acid by fatty acid amide hydrolase (FAAH) and can serve as a substrate, although with low affinity, for cyclooxygenase-2 (COX-2), 12- and 15-lipoxygenases (LOXs) and P450.

Figure 2. Anandamide and its analogs did not share discriminative stimulus effects with Δ^9 -THC in C57BL/6J mice

Animals Δ^9 -THC (3.2 mg/kg i.p.) from vehicle (18 parts saline, 1 part Tween 80, and 1 part propylene glycol) under a fixed ratio 30 schedule of milk delivery. Operant sessions consisted of consecutive cycles. Each cycle began with a 15-min timeout followed by a 5-min period of operant responding. Increasing doses of drugs were administered at the beginning of each cycle, thereby permitting determination of a full dose-response curve in a single session. See [194] for experimental details. Abscissae: dose in milligram per kilogram of body weight or vehicle (VEH). Ordinates: mean ($\pm$ S.E.M.) Δ^9 -THC-appropriate responding (top) and mean ($\pm$ S.E.M.) response rate (responses per second). Dashed lines show the 95% confidence limits of the vehicle control response rate determined from ten vehicle training sessions; symbols outside the confidence limits are considered significantly different from control.

Figure 3. Rimonabant antagonized the hypothermic effects of CP55940, WIN55212-2, and Δ^9 -THC but not anandamide and its analogs in C57BL/6J mice

Mice ($n = 6-8$) received vehicle (18 parts saline, 1 part Tween 80, and 1 part propylene glycol) or a rimonabant dose (3.2-32 mg/kg) followed 20 min later by cumulative doses of cannabinoid agonists every 20 min. The relative potency of the agonists to produce hypothermia was similar to their relative potency in producing discriminative stimulus or rate-decreasing effects (See Figure 2). All doses of rimonabant produced significant and dose-dependent antagonism of CP55940, WIN55212-2, and Δ^9 -THC (left panels), as evidenced by significant increases in the control ED_{50} of each agonist (i.e. the 95% confidence limits of the potency ratio did not include 1; the potency ratio was defined as the ED_{50} in the presence of a dose of rimonabant divided by the control ED_{50}). By contrast, rimonabant did not significantly antagonize the hypothermic effects of arachidonylcyclopropylamide (ACPA), anandamide, and methanandamide (right panels). Abscissae: dose in milligram per kilogram of body weight or vehicle (VEH). Ordinates: mean ($\pm$ S.E.M.) decrease in body temperature ($^{\circ}\text{C}$) relative to control determined immediately prior to drug administration.