Published in final edited form as:
Pharmacol Res. 2007 November ; 56(5): 360–366.

Enhancement of endocannabinoid signaling and the pharmacotherapy of depression

Regina A. Mangieri and **Daniele Piomelli**
Department of Pharmacology and Center for Drug Discovery, The University of California, Irvine, California 92697 USA

Abstract

Cannabinoids are well known modulators of mood and emotional behavior. Current research supports a role for endocannabinoid signaling in the treatment of depression. Changes in levels of the cannabinoid CB_1 receptor, or the endogenous CB_1 receptor ligands, anandamide and 2-AG, are observed both in humans suffering from depression and in animal models of depression, and experimental manipulation of CB_1 receptor signaling has also been shown to affect emotional reactivity in rodents. Importantly, inhibitors of anandamide inactivation have demonstrated efficacy in enhancing stress-coping and mood-related behavior. This article will review these areas of research, highlighting the potential of endocannabinoid metabolism modulators as therapeutics for the treatment of depression.

Introduction

In addition to producing a well-described series of somatic effects – such as decreased motor activity, increased feeding, and analgesia (for review see Mackie 2006 [1]) – CB_1 cannabinoid receptors also appear to play important, albeit complex, roles in neuropsychiatric disease. Emerging evidence indicates that modulation of CB_1 receptor signaling may be useful for the treatment of several mental disorders, such as depression, anxiety, and addiction. This review will focus on the literature suggesting a role for modulation of endogenous cannabinoid (endocannabinoid) signaling in the treatment of depression. Excellent reviews on the contribution of the endocannabinoids to anxiety and addiction have been recently published [2,3]

Depression is a psychiatric disorder characterized in humans by the core symptoms of depressed mood and/or loss of pleasure or interest in most activities (anhedonia) [4]. Other characteristics include, but are not limited to, changes in body weight, sleeping patterns, psychomotor behavior, energy level, and cognitive functioning [4]. The overlap between the physiological functions altered by depression and those affected by cannabinoid receptor signaling is striking, and suggests that activation of this system may have important effects on the regulation of mood disorders. In fact, prolonged cannabis consumption and cannabis withdrawal in people are often associated with depression, but whether marijuana use

Corresponding Author: Daniele Piomelli, University of California, Irvine Department of Pharmacology 3101, Gillespie NRF Irvine, CA 92697, Phone: (949) 824-6180, Fax: (949) 824-6305, piomelli@uci.edu.

Disclosure
Dr. Piomeli is a co-founder of, and consultant for Kadmus Pharmaceuticals, Inc., which is currently developing URB597 (KDS-4103).

contributes to the development of this disorder is still a matter of debate (for review see Degenhardt *et al*. [5]).

These considerations have prompted numerous researchers to investigate the endocannabinoid system as it relates to depression and mood disorders. There is now persuasive evidence from several areas of research, outlined in this article, which suggests a role for the endocannabinoid system in the normal regulation of mood, as well as in the pathogenesis and treatment of depression and other stress-related disorders. First of all, studies of both animals and humans suggest that alterations in endocannabinoid signaling may participate in depression-related behaviors. Moreover, direct modulation of cannabinoid CB_1 receptor signaling, by natural or synthetic agonists, as well as antagonists, can produce effects on stress-responses and mood-related behavior. Finally, several enzymes responsible for the metabolism of endocannabinoids have been identified, leading to the development of drugs that indirectly enhance cannabinoid receptor signaling by blocking endocannabinoid deactivation. These pharmacological tools have substantiated the notion that augmentation of endogenous cannabinoid signaling may promote stress-coping behavior, both under normal and pathophysiological conditions. Together, the evidence indicates that the endogenous cannabinoid system is a modulator of mood states and a promising target for the treatment of stress-related mood disorders such as depression.

The endogenous cannabinoid system

The best characterized endogenous cannabinoid ligands, arachidonoylethanolamide [6,7] (anandamide) and *sn*-2-arachidonoylglycerol (2-AG) [8–10] are produced in an activity-dependent manner and appear to locally modulate synaptic transmission in the nervous system *via* presynaptic activation of the $G\alpha_{i/o}$-protein coupled cannabinoid CB_1 receptor [11]. Anandamide and 2-AG also bind to and activate the $G\alpha_{i/o}$-protein coupled cannabinoid CB_2 receptor [12], but the possible roles of this receptor in the central nervous system (CNS) are only beginning to be understood [13–15].

The pattern of distribution of CB_1 receptors is reflective of the proposed roles for this system in the modulation of pain perception, affective states, stress responses, motor activity, and cognitive functioning [16]. CB_1 is found at highest concentrations in the hippocampus, basal ganglia, neocortex, cerebellum and anterior olfactory nucleus [17–19]. Moderate levels of the receptor are also present in the basolateral amygdala, hypothalamus, and midbrain periaqueductal gray [17–20]. Initially, the CB_2 receptor was found to be localized predominantly in peripheral tissues and particularly in immune cells, but recent articles have reported CB_2 mRNA expression in the brainstem [13] and CB_2 immunohistochemical staining throughout the brain [21].

Unlike many traditional neurotransmitters, the endocannabinoid ligands are lipid-derived amphipathic messengers that are not stored in vesicles. Rather, they appear to be produced from precursor components within the cellular membrane. In the best characterized synthesis pathway, the anandamide precursor, *N*-arachidonoyl-phosphatidylethanolamine (NAPE), is formed by an *N*-acyltransferase (NAT)-catalyzed transfer of an arachidonic acid moiety from the s*n*-1 position of phosphatidylcholine (PC) to the amine group of phosphatidylethanolamine (PE) [22]. NAPEs are then cleaved by a NAPE-specific phosopholipase D (NAPE-PLD), an isoform of which has recently been cloned [23], to produce anandamide. Alternatively, NAPEs can be hydrolyzed by a phopholipase C (PLC) enzyme to generate phosphoanandamide, which is then dephosphorylated by a phosphatase, such as the protein tyrosine phosphatase PTPN22, to yield anandamide [24]. The biological deactivation of anandamide is likely a two-step process [25], whereby the lipid mediator is transported into cells by a presently uncharacterized

entity, and then hydrolyzed by the membrane-bound enzyme fatty-acid amide hydrolase (FAAH) to form ethanolamine and arachidonic acid [26,27].

Two main biochemical pathways exist, which can potentially generate 2-AG. The 2-AG precursor, 1,2-diacyl-s*n*-glycerol (DAG), can be formed from phosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) by the action of a PI-specific PLC [16]. Two isoforms, α and β, of the enzyme diacylglycerol lipase (DGL) have been shown to form 2-AG from DAG (*28*). An alternate pathway is possible, whereby 2-AG could be formed by the sequential actions of phospholipase A_1 and lysophospholipase C enzymes [16,29–31]. The primary route for 2-AG hydrolysis in neurons is afforded by the enzyme monoacylglycerol lipase (MGL) [32]. Recently, a pharmacologically distinct monoglyceride lipase activity in microglial cells has been reported [33].

In order to understand better the role endocannabinoids might have in CB_1-regulated behaviors, a number of pharmacological tools, which target events in endocannabinoid metabolism, have been developed. Anandamide deactivation is prevented by the transport inhibitors AM404 [25], UCM707 [34], OMDM-1 and OMDM-2 [35], and VDM11 [36], and the FAAH-selective anandamide hydrolysis inhibitors URB597 [37–39] and OL-135 [40]. 2-AG hydrolysis is blocked by the MGL inhibitor URB602 [41,42]. Pharmacological inhibition of endocannabinoid deactivation has been shown to produce anxiolytic, analgesic, and antidepressant-like effects [37,43–47]. The antidepressant-like effects of anandamide deactivation inhibitors will be discussed in detail later in the present article.

Cannabinoid alterations during depression

Limited, but compelling evidence indicates that the endocannabinoid system is altered during stress-related states in both rodents and humans. The chronic mild or chronic unpredictable stress (CMS/CUS) protocol are two related models of depression that produce sequelae reminiscent of those observed in humans afflicted with the disease. These include, among others, a reduction in body weight gain and ingestion of palatable foods [48]. In rats subjected to 3 weeks of CUS, Hill and colleagues found a significant reduction of 2-AG content, as well as levels of CB_1 receptor protein in the hippocampus [49]. Stressed animals also showed impairment of reversal learning in the Morris water maze, which was corrected by administration of the cannabinoid agonist HU 210, suggesting that this effect was due to decreased endocannabinoid signaling. Similarexperiments in our lab have shown that after 10 weeks of CMS, CB_1 receptor mRNA is increased in the prefrontal cortex and decreased in the rat midbrain [44]. Anandamide levels in the hippocampus, prefrontal cortex, midbrain, thalamus, and striatum were not significantly altered in these studies. 2-AG was similarly unchanged in the hippocampus, prefrontal cortex, midbrain, and striatum, but was reduced in the thalamus of stress-exposed rats.

Changes in endocannabinoid signaling have also been documented in depressed human subjects. In a study of 20 human subjects, Hungund *et al*. found an increase in both CB_1 receptor mRNA and CB_1 receptor-stimulated [^{35}S]$GTP_\gamma S$ binding in the dorsolateral prefrontal cortex of subjects with a life-time diagnosis of major depression who committed suicide, compared to normal controls (matched by age, sex, and postmortem interval) who died by accident or natural causes [50]. Miller and colleagues reported reduced serum 2-AG levels in drug-free females diagnosed with major depression compared to demographically-matched controls, with levels of 2-AG negatively correlated to the duration of the depressive episode [51]. In the latter study, serum anandamide was not associated with major depression, but was negatively correlated with measures of anxiety.

The results of these studies of both rodents and humans provide evidence that endocannabinoid signaling is changed – at least in some brain regions and, perhaps, in the periphery – during

depression. The alterations observed in the hippocampus, prefrontal cortex, and thalamus are of particular interest, given the likely involvement of these neural structures in the regulation of emotion [52].

Effects of direct CB_1 receptor modulation on emotional behavior

In humans, Δ^9-THC, the natural cannabinoid agonist that is the major psychoactive component of marijuana [53], produces subjective feelings of relaxation and euphoria, but also promotes anxiety and dysphoria in a context- and dose-dependent manner [54–58]. Similarly, when administered to rodents, exogenous cannabinoid agonists produce mixed effects on mood-related behavior. Low doses of cannabinoid agonists are usually anxiolytic, while moderate to high doses are anxiogenic, but these dose-dependent effects are also contingent on other factors, including strain, age, sex, environment and previous experience with the drug [59]. In mice, Δ^9-THC produced anxiolytic effects in the light/dark box at a dose of 0.3 mg-kg^{-1}, i.p., but at 5 mg-kg^{-1}, i.p., induced anxiogenic effects [60,61]. HU 210, a highly potent cannabinoid receptor agonist, at a dose of 0.1 mg-kg^{-1}, i.p., has also been reported to produce anxiogenic effects in the defensive-withdrawal test after acute administration [62], but, when this same dose was administered for 10 days it exerted antidepressant-like effects in the novelty-suppressed feeding and forced swim tests [63]. Comparable dose- and context-dependent effects on mood-related behavior in the elevated-plus maze and social interaction tests have been noted following treatment with another synthetic cannabinoid agonist, CP 55,940 [64, 65].

Data from experiments with CB_1 knockout mice suggest that prevention of cannabinoid signaling either increases or has no effect on anxiety- and depression-related behaviors, depending on the conditions of the test [66–71]. Notably, in these studies, CB_1 knockout mice displayed increased anxiety-like behavior compared to wild-type controls under conditions that are stressful to the animals (i.e., high light, novel environment). Additionally, CB_1 receptor knockout mice have increased sensitivity to develop anhedonia in the CUS model of depression [67], and display several other behavioral responses that are similar to the symptoms of melancholic depression (reviewed in Hill and Gorzalka [72]). Likewise, several researchers [73–77] have reported that administration of the CB_1 receptor antagonists SR141716 (rimonabant) and AM251 produced anxiogenic-like effects. By contrast, few groups reported anxiolytic- and antidepressant-like effects of CB_1 receptor antagonists [78,79]. However, in clinical trials of rimonabant for the treatment of obesity, anxiety and depression are among the most frequent adverse events reported [80–84]. Together, these studies suggest that CB_1 receptor signaling is important for coping behavior, especially during intense or prolonged stress.

Indirect modulation of CB_1 receptor signaling as a strategy for depression pharmacotherapy

As described in the previous section, changes in endocannabinoid activity might occur during depression in animal models and, possibly, in humans. Furthermore, direct activation or reduction of CB_1 receptor signaling has important effects on mood and stress-related behaviors. These findings raise the intriguing possibility that modulation of endogenous cannabinoid signaling could be a useful target for depression therapy. Indeed, enhancement of endocannabinoid signaling by pharmacological inhibitors of anandamide degradation has been shown to modulate stress-related behavior in assays for antidepressant-like drug activity – the forced swim test (FST) and tail suspension test (TST) – and in a rodent model of depression – chronic mild stress (CMS) (Table 1). The anandamide transport inhibitor, AM404, at a dose of 5 mg-kg^{-1}, was reported to decrease immobility time in the rat FST [45]. Likewise, the fatty-acid amide hydrolase inhibitor, URB597 (0.1 and 0.3 mg-kg^{-1}), decreased immobility –

presumably by increasing swimming behavior – in the rat FST, and also increased struggling behavior in the mouse TST [85]. These effects of URB597 in the FST and TST were sustained after 4 days of repeated dosing. In each of these tests, the antidepressant-like activity of AM404 or URB597 was prevented by preadministration of a selective CB_1 receptor antagonist.

Given that symptoms of anxiety are often present during depression [4], it is noteworthy that anandamide deactivation inhibitors also appear to have anxiolytic-like effects. Administration of URB597 decreased isolation-induced ultrasonic vocalizations in rat pups, and increased the time spent in the open arms of the elevated zero and plus mazes [37,47,86,87]. Similarly, AM404 dose-dependently reduced isolation-induced ultrasonic vocalizations in rat pups, and increased the time spent in the open arms of the elevated plus maze or in the open field during the defensive withdrawal test [43].

However, it appears that the effects of inhibition of anandamide deactivation on stress-coping behaviors are sensitive to environmental conditions. In a recent report, Naidu and colleagues failed to find a reduction of immobility in the TST or an increase in the percentage of time spent in the open arms in the elevated plus maze in $FAAH^{-/-}$ mice or in wild type mice treated with URB597 when conducted under normal laboratory lighting [47]. However, when they adopted lighting conditions similar to those used by Patel and Hillard in the elevated plus maze (shadowed closed arms and brightly lit open arms), or Gobbi and colleagues in the TST (dimmed room with bright light focused on the tail of the mouse), they did observe anxiolytic and antidepressant-like effects of FAAH deletion or inhibition [47]. The reported sensitivity of the anxiolytic- and antidepressant-like effects of URB597 to the lighting conditions is consistent with recent findings in our lab, which show that the anxiolytic-like effect of URB597 in the elevated plus maze varies with experimental context [59].

It is important to note that both the tail suspension and forced swim tests are only assays for antidepressant-like drug activity, not models of depression. In the reports cited above, the experiments were performed in undiseased animals, demonstrating an enhancement of active stress-coping behavior by URB597 or AM404 in a manner similar to standard antidepressant drugs during normal physiological conditions, but under specific environmental contexts. The ability of inhibitors of anandamide degradation to regulate stress-related behaviors under pathophysiological conditions should be more indicative of their efficacy in the treatment of depression. In the CMS model, administration of URB597 for 5 weeks at a dose of 0.3 mg-kg^{-1} reversed chronic stress-induced reductions in sucrose consumption and in body weight gain [44]. In this same study, treatment with URB597 also opposed the increases in CB_1 mRNA expression in the prefrontal cortex and midbrain that were observed after 10 weeks of CMS. The magnitude and time course for the antidepressant-like effect of URB597 in this study was comparable to that seen in the treatment of depression with the known antidepressant compound, imipramine.

These findings are important because they demonstrate, for the first time, the ability of an anandamide deactivation inhibitor to reverse behavioral symptoms observed in a model of depression with high construct and face validity. It is important to note though, that alterations in 2-AG are observed both in depressed humans and in animal models of depression, and the significance of these changes are unclear.

FAAH inhibitors have proven to be valuable tools for investigating the role of anandamide in mood disorders, and DGL and MGL inhibitors will no doubt further elucidate the interaction between endogenous cannabinoid signaling and stress-related behaviors. For example, the MGL inhibitor, URB602, when injected locally into the dorsolateral periaqueductal grey of the midbrain, produced an enhancement of stress-induced analgesia, demonstrating a role for 2-AG in a specific stress-coping response [41]. Inhibition of MGL has also identified 2-AG as

a mediator of synaptic plasticity in the hippocampus [42], a structure likely involved in the effects of chronic stress and antidepressant treatment on behavior [52,88]. Unfortunately, URB602 has low potency and cannot be administered systemically to study the effects of global *in vivo* modulation of 2-AG on stress-coping behavior. As inhibitors of DGL and MGL are developed and tested in behavioral models of emotional reactivity, we will have a better understanding of the functions of both endocannabinoid signaling molecules, perhaps each with distinct roles in stress-coping and mood disorders.

Conclusions

Recent work has provided impetus to believe that manipulations of the endogenous cannabinoid system could be useful to alleviate symptoms of depression. Alterations in the endogenous lipids anandamide and 2-AG, as well as the CB_1 receptor, are observed during human and experimental models of depression. However, direct modifications of CB_1 receptor signaling have variable effects on mood-related behavior. Recent advances in the understanding of endocannabinoid biochemistry have made it possible to study the behavioral effects of pharmacological manipulation of levels of the endocannabinoid signaling molecules. Notably, enhancement of CB_1 receptor signaling *via* systemic blockade of anandamide hydrolysis appears to have efficacy in reversing symptoms of depression, and local inhibition of 2-AG degradation suggests that this molecule also could be important for the regulation of stress-coping behavior. These findings highlight the potential for endocannabinoid metabolism modulators as novel therapeutics for the treatment of depression.

Acknowledgements

This work was supported by grants from the National Institute on Drug Abuse (NIDA) DA12413, DA12447, and DA07318.

References

1. Mackie K. Cannabinoid receptors as therapeutic targets. Annu Rev Pharmacol Toxicol 2006;46:101–22. [PubMed: 16402900]
2. Viveros MP, Marco EM, File SE. Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav 2005;81:331–42. [PubMed: 15927244]
3. Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 2006;29:225–32. [PubMed: 16483675]
4. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV. American Psychiatric Association; Washington, DC: 1994.
5. Degenhardt L, Hall W, Lynskey M. Exploring the association between cannabis use and depression. Addiction 2003;98:1493–504. [PubMed: 14616175]
6. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9. [PubMed: 1470919]
7. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 1994;372:686–91. [PubMed: 7990962]
8. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 1995;50:83–90. [PubMed: 7605349]
9. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 1995;215:89–97. [PubMed: 7575630]
10. Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature 1997;388:773–8. [PubMed: 9285589]

11. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 2003;83:1017–66. [PubMed: 12843414]
12. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5. [PubMed: 7689702]
13. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, et al. Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 2005;310:329–32. [PubMed: 16224028]
14. Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, et al. Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann N Y Acad Sci 2006;1074:514–36. [PubMed: 17105950]
15. Ishiguro H, Iwasaki S, Teasenfitz L, Higuchi S, Horiuchi Y, Saito T, et al. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. Pharmacogenomics J. 2006
16. Piomelli D. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 2003;4:873–84. [PubMed: 14595399]
17. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 1991;11:563–83. [PubMed: 1992016]
18. Matsuda LA, Bonner TI, Lolait SJ. Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 1993;327:535–50. [PubMed: 8440779]
19. Glass M, Dragunow M, Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 1997;77:299–318. [PubMed: 9472392]
20. Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, et al. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 2001;21:9506–18. [PubMed: 11717385]
21. Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, et al. Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 2006;1071:10–23. [PubMed: 16472786]
22. Jin XH, Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Discovery and characterization of a Ca2+-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners. J Biol Chem 2007;282:3614–23. [PubMed: 17158102]
23. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 2004;279:5298–305. [PubMed: 14634025]
24. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A 2006;103:13345–50. [PubMed: 16938887]
25. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli P. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 1997;277:1094–7. [PubMed: 9262477]
26. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83–7. [PubMed: 8900284]
27. Désarnaud F, Cadas H, Piomelli D. Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. J Biol Chem 1995;270:6030–5. [PubMed: 7890734]
28. Bisogno T, Howell F, Williams G, Minassi A, Cascio M, Ligresti A, et al. Cloning the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. The Journal of Cell Biology 2003;163:463–468. [PubMed: 14610053]
29. Farooqui AA, Rammohan KW, Horrocks LA. Isolation, characterization, and regulation of diacylglycerol lipases from the bovine brain. Ann N Y Acad Sci 1989;559:25–36. [PubMed: 2774400]
30. Higgsand HN, Glomset JA. Identification of a phosphatidic acid-preferring phospholipase A1 from bovine brain and testis. Proc Natl Acad Sci U S A 1994;91:9574–8. [PubMed: 7937808]
31. Pete MJ, Ross AH, Exton JH. Purification and properties of phospholipase A1 from bovine brain. J Biol Chem 1994;269:19494–500. [PubMed: 8034719]

32. Dinh TP, Kathuria S, Piomelli D. RNA Interference Suggests a Primary Role for Monoacylglycerol Lipase in the Degradation of the Endocannabinoid 2-Arachidonoylglycerol. Mol Pharmacol 2004 Nov;66(5):1260–4. [PubMed: 15272052]
33. Muccioli GG, Xu C, Odah E, Cudaback E, Cisneros JA, Lambert DM, et al. Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells. J Neurosci 2007;27:2883–9. [PubMed: 17360910]
34. Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, de Lago E, et al. Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: comparison with effects on fatty acid amidohydrolase. J Med Chem 2003;46:1512–22. [PubMed: 12672252]
35. Ortar G, Ligresti A, De Petrocellis L, Morera E, Di Marzo V. Novel selective and metabolically stable inhibitors of anandamide cellular uptake. Biochem Pharmacol 2003;65:1473–81. [PubMed: 12732359]
36. De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V. 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 2000;483:52–6. [PubMed: 11033355]
37. Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, et al. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 2003;9:76–81. [PubMed: 12461523]
38. Clapper JR, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. The fatty-acid amide hydrolase inhibitor URB597 does not affect triacylglycerol hydrolysis in rat tissues. Pharmacol Res 2006;54:341–4.10.1016/j.phrs.2006.06.008 [PubMed: 16935521]
39. Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton T, et al. Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev 2006;12(1):21–38. [PubMed: 16834756]
40. Boger DL, Miyauchi H, Du W, Hardouin C, Fecik RA, Cheng H, et al. Discovery of a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. J Med Chem 2005;48:1849–56. [PubMed: 15771430]
41. Hohmann AG, Suplita RL II, Bolton NM, Neely MH, Fegley D, Mangieri R, et al. An endocannabinoid mechanism for stress-induced analgesia. Nature 2005 Jun 23;435(7045):1108–12. [PubMed: 15973410]
42. Makara JK, Mor M, Fegley D, Szabo SI, Kathuria S, Astarita G, et al. Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. Nat Neurosci 2005;8:1139–41. [PubMed: 16116451]
43. Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V, et al. Anxiolytic-Like Properties of the Anandamide Transport Inhibitor AM404. Neuropsychopharmacology 2006 Dec;31 (12):2652–9. [PubMed: 16541083]
44. Bortolato M, Mangieri RA, Fu J, Kim J, Arguello O, Duranti A, et al. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biological Psychiatry. 200710.1016/j.biopsych.2006.12.001
45. Hill MN, Gorzalka BB. Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. Eur Neuropsychopharmacol 2005;15:593–9. [PubMed: 15916883]
46. La Rana G, Russo R, Campolongo P, Bortolato M, Mangieri RA, Cuomo V, et al. Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404. J Pharmacol Exp Ther 2006 Jun;317(3):1365–71. [PubMed: 16510698]
47. Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH. Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. Psychopharmacology (Berl) 2007 May;192 (1):61–70. [PubMed: 17279376]
48. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl) 1997;134:319–29. [PubMed: 9452163]
49. Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, et al. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 2005;30:508–15. [PubMed: 15525997]

50. Hungund BL, Vinod KY, Kassir SA, Basavarajappa BS, Yalamanchili R, et al. Upregulation of CB1 receptors and agonist-stimulated [35S]GTPgammaS binding in the prefrontal cortex of depressed suicide victims. Mol Psychiatry 2004;9:184–90. [PubMed: 14966476]
51. Miller, GE.; Hill, MN.; Ho, WSV.; Gorzalka, BB.; Hillard, CJ. Bidirectional alterations in serum endocannabinoids in minor and major depression. Society for Neuroscience; Washington, D. C: 2005.
52. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006;7:137–51. [PubMed: 16429123]
53. Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–1647.
54. Dannon PN, Lowengrub K, Amiaz R, Grunhaus L, Kotler M. Comorbid cannabis use and panic disorder: short term and long term follow-up study. Hum Psychopharmacol 2004;19:97–101. [PubMed: 14994319]
55. Green B, Kavanagh D, Young R. Being stoned: a review of self-reported cannabis effects. Drug Alcohol Rev 2003;22:453–60. [PubMed: 14660135]
56. Hall W, Solowij N. Adverse effects of cannabis. Lancet 1998;352:1611–6. [PubMed: 9843121]
57. Hollister LE. Health aspects of cannabis. Pharmacol Rev 1986;38:1–20. [PubMed: 3520605]
58. Patton GC, Coffey C, Carlin JB, Degenhardt L, Lynskey M, Hall W. Cannabis use and mental health in young people: cohort study. Bmj 2002;325:1195–8. [PubMed: 12446533]
59. Bortolato, M.; Piomelli, D. The endocannabinoid system and anxiety responses. Griebel, G., editor. Elsevier; 2007.
60. Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R. Behavioural and biochemical evidence for interactions between Delta9- tetrahydrocannabinol and nicotine. Br J Pharmacol 2002;135:564–78. [PubMed: 11815392]
61. Berrendero F, Maldonado R. Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl) 2002;163:111–7. [PubMed: 12185408]
62. Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GJ, et al. Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. J Pharmacol Exp Ther 1996;276:56–64. [PubMed: 8558457]
63. Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, et al. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J Clin Invest 2005;115:3104–3116. [PubMed: 16224541]
64. Marco EM, Perez-Alvarez L, Borcel E, Rubio M, Guaza C, Ambrosio E, et al. Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav Pharmacol 2004;15:21–7. [PubMed: 15075623]
65. Genn RF, Tucci S, Marco EM, Viveros MP, File SE. Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test. Pharmacol Biochem Behav 2004;77:567–73. [PubMed: 15006468]
66. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–4. [PubMed: 12152079]
67. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology (Berl) 2002;159:379–87. [PubMed: 11823890]
68. Haller J, Bakos N, Szirmay M, Ledent C, Freund TF. The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. Eur J Neurosci 2002;16:1395–8. [PubMed: 12405999]
69. Alberich Jorda M, Verbakel SE, Valk PJ, Vankan-Berkhoudt YV, Maccarrone M, Finazzi-Agro A, et al. Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to the endocannabinoid 2-arachidonoylglycerol. Blood 2002;99:2786–93. [PubMed: 11929767]
70. Uriguen L, Perez-Rial S, Ledent C, Palomo T, Manzanares J. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 2004;46:966–73. [PubMed: 15081793]

71. Haller J, Varga B, Ledent C, Barna I, Freund TF. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. Eur J Neurosci 2004;19:1906–12. [PubMed: 15078564]
72. Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol 2005;16:333–52. [PubMed: 16148438]
73. Haller J, Varga B, Ledent C, Freund TF. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. Behav Pharmacol 2004;15:299–304. [PubMed: 15252281]
74. Arevalo C, de Miguel R, Hernandez-Tristan R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. Pharmacol Biochem Behav 2001;70:123–31. [PubMed: 11566149]
75. Navarro M, Hernández E, Muñoz RM, del Arco I, Villanúa MAM, Carrera RA, et al. Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. NeuroReport 1997;8:491–6. [PubMed: 9080435]
76. Rodgers RJ, Evans PM, Murphy A. Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. Behav Pharmacol 2005;16:405–13. [PubMed: 16148445]
77. McGregor IS, Dastur FN, McLellan RA, Brown RE. Cannabinoid modulation of rat pup ultrasonic vocalizations. Eur J Pharmacol 1996;313:43–9. [PubMed: 8905327]
78. Shearman LP, Rosko KM, Fleischer R, Wang J, Xu S, Tong XS, et al. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. Behav Pharmacol 2003;14:573–82. [PubMed: 14665974]
79. Griebel G, Stemmelin J, Scatton B. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry 2005;57:261–7. [PubMed: 15691527]
80. Despres JP, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med 2005;353:2121–34. [PubMed: 16291982]
81. Gelfand EV, Cannon CP. Rimonabant: a selective blocker of the cannabinoid CB1 receptors for the management of obesity, smoking cessation and cardiometabolic risk factors. Expert Opin Investig Drugs 2006;15:307–15.
82. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA 2006;295:761–75. [PubMed: 16478899]
83. Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet 2006;368:1660–72. [PubMed: 17098084]
84. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 2005;365:1389–97. [PubMed: 15836887]
85. Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, et al. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci U S A 2005;102:18620–5. [PubMed: 16352709]
86. Hill MN, Karacabeyli ES, Gorzalka BB. Estrogen recruits the endocannabinoid system to modulate emotionality. Psychoneuroendocrinology 2007 May;32(4):350–7. [PubMed: 17391861]
87. Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther 2006;318:304–11. [PubMed: 16569753]
88. Dranovsky A, Hen R. Hippocampal neurogenesis: regulation by stress and antidepressants. Biol Psychiatry 2006;59:1136–43. [PubMed: 16797263]

Table 1

Effects of anandamide deactivation inhibitors in rodent tests for antidepressant-like drug activity

Test/Model	Drug (mg-kg^{-1})	Behavioral Effect	Reference
Forced Swim Test	AM404 (5)	↓immobility	(43)
	URB 597 (0.1, 0.3)	↓immobility/↑swimming	(83)
Tail Suspension Test	URB 597 (0.1, 0.3)	↓immobility*	(83), (45)*
Chronic Mild Stress	URB 597 (0.3)	Reversed reduction in body weight gain	(42)
		Reversed reduction in sucrose consumption	(42)

* In Naidu *et al.* (45) a reduction of immobility was only found when lighting conditions were the same as those used by Gobbi *et al.* (83); see text for details.