Enhancement of endocannabinoid signaling and the pharmacotherapy of depression

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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 CB1 receptor or the endogenous CB1 receptor ligands, anandamide and 2-AG, are observed both in humans suffering from depression and in animal models of depression, and experimental manipulation of CB1 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.

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Keywords: Endocannabinoid; Anandamide; 2-AG; Depression

Contents

1. Introduction ................................................. 360
2. The endogenous cannabinoid system .............................................................. 361
3. Cannabinoid alterations during depression .................................................. 361
4. Effects of direct CB1 receptor modulation on emotional behavior ............... 362
5. Indirect modulation of CB1 receptor signaling as a strategy for depression pharmacotherapy .................................................. 362
6. Conclusions ............................................... 363

Disclosure .............................................................................................................. 364
Acknowledgements ..................................................................................................... 364
References ............................................................................................................. 364

1. 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 [1]) – CB1 cannabinoid receptors also appear to play important, albeit complex, roles in neuropsychiatric disease. Emerging evidence indicates that modulation of CB1 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 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 CB1 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 endocannabinoid system is a modulator of mood states and a promising target for the treatment of stress-related mood disorders such as depression.

2. The endogenous cannabinoid system

The best characterized endogenous cannabinoid ligands, arachidonylethanolamide [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 CB1 receptor [11]. Anandamide and 2-AG also bind to and activate the G\(\alpha_{i/o}\)-protein coupled cannabinoid CB2 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 CB1 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]. CB1 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 CB2 receptor was found to be localized predominantly in peripheral tissues and particularly in immune cells, but recent articles have reported CB2 mRNA expression in the brainstem [13] and CB2 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-acetyltransferase (NAT)-catalyzed transfer of an arachidonic acid moiety from the sn-1 position of phosphatidylethanolamine (PC) to the amine group of phosphatidylethanolamine (PE) [22]. NAPEs are then cleaved by a NAPE-specific phospholipase D (NAPE-PLD), an isomeric of which has recently been cloned [23], to produce anandamide. Alternatively, NAPEs can be hydrolyzed by a phospholipase 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-sn-glycerol (DAG), can be formed from phosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) 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\(_2\) 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 CB1-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.

3. 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 et al. 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 impaired learning of aversive 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. Similar experiments 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 $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ 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 et al. 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].

4. Effects of direct CB$_1$ receptor modulation on emotional behavior

In humans, $\Delta^9$-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, $\Delta^9$-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.

5. 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 et al. 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 co-workers 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 systematically 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.

### 6. 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.

### Table 1

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

<table>
<thead>
<tr>
<th>Test/model</th>
<th>Drug (mg kg^{−1})</th>
<th>Behavioral effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forced swim test</td>
<td>AM404 (5)</td>
<td>↓ Immobility</td>
<td>[45]</td>
</tr>
<tr>
<td>Tail suspension test</td>
<td>URB597 (0.1, 0.3)</td>
<td>↓ Immobility↑ swimming</td>
<td>[85]</td>
</tr>
<tr>
<td>Chronic mild stress</td>
<td>URB597 (0.3)</td>
<td>Reversed reduction in body weight gain</td>
<td>[44]</td>
</tr>
</tbody>
</table>

a In Naidu et al. [47] a reduction of immobility was only found when lighting conditions were the same as those used by Gobbi et al. [85]; see text for details.
Disclosure

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

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