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Functional Redundancy Between Canonical Endocannabinoid Signaling Systems in the Modulation of Anxiety

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ABSTRACT

BACKGROUND: Increasing the available repertoire of effective treatments for mood and anxiety disorders represents a critical unmet need. Pharmacological augmentation of endogenous cannabinoid (eCB) signaling has been suggested to represent a novel approach to the treatment of anxiety disorders; however, the functional interactions between two canonical eCB pathways mediated via anandamide (*N*-arachidonylethanolamine [AEA]) and 2-arachidonoylglycerol (2-AG) in the regulation of anxiety are not well understood.

METHODS: We utilized pharmacological augmentation and depletion combined with behavioral and electrophysiological approaches to probe the role of 2-AG signaling in the modulation of stress-induced anxiety and the functional redundancy between AEA and 2-AG signaling in the modulation of anxiety-like behaviors in mice.

RESULTS: Selective 2-AG augmentation reduced anxiety in the light/dark box assay and prevented stress-induced increases in anxiety associated with limbic AEA deficiency. In contrast, acute 2-AG depletion increased anxiety-like behaviors, which was normalized by selective pharmacological augmentation of AEA signaling and via direct cannabinoid receptor 1 stimulation with Δ^9-tetrahydrocannabinol. Electrophysiological studies revealed 2-AG modulation of amygdala glutamatergic transmission as a key synaptic correlate of the anxiolytic effects of 2-AG augmentation.

CONCLUSIONS: Although AEA and 2-AG likely subserve distinct physiological roles, a pharmacological and functional redundancy between these canonical eCB signaling pathways exists in the modulation of anxiety-like behaviors. These data support development of eCB-based treatment approaches for mood and anxiety disorders and suggest a potentially wider therapeutic overlap between AEA and 2-AG augmentation approaches than was previously appreciated.

Keywords: Amygdala, Anxiety, 2-Arachidonoylglycerol, JZL184, MAGL inhibition, Stress

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Mood and anxiety disorders as a group are the most prevalent mental health condition (1). Between 1990 and 2013, the number of people experiencing anxiety and/or depression increased by nearly 50%, from 416 million to 615 million (2). The high prevalence of mood and anxiety disorders, including posttraumatic stress disorder, panic disorder, and generalized anxiety disorders, underscores the critical need for innovative treatment approaches for this class of disorders. The current primary treatment for these disorders produces a delayed onset of action with modest therapeutic benefits (3), while long-term treatment with fast-acting benzodiazepines can cause potentially significant adverse effects (4–6).

The endogenous cannabinoid (eCB) system is a retrograde lipid signaling system that is implicated in the regulation of multiple physiological functions in the nervous system (7,8). Anandamide (*N*-arachidonylethanolamine [AEA]) and 2-arachidonoylglycerol (2-AG) are two well-studied eCBs that exert biological effects via activation of types 1 and 2 cannabinoid receptors (CB_1R and CB_2R) (9,10). Preclinical studies support the role of the eCB system as a modulator of unconditioned anxiety-related behaviors, depressive-like behaviors, and extinction of fear memories (11–15). Although significant literature supports preclinical efficacy of AEA augmentation to treat anxiety-related disorders (11,14,16–23), the therapeutic potential of 2-AG augmentation is only beginning to be elucidated. 2-AG is the most abundant eCB in the brain, and clinical studies have demonstrated reduced peripheral 2-AG levels in patients with posttraumatic stress disorder and with major depression (24,25). Consistent with these findings, we and others recently showed that genetic deficiency in 2-AG signaling is sufficient to induce anxious and depressive-like behavioral states (26,27). Similarly, recent studies have demonstrated that augmenting 2-AG signaling can reduce anxiety-like behaviors (28–30). Despite these data, a central question remains as to whether these two canonical

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eCB signaling systems, AEA and 2-AG, exhibit redundancy in their effect on anxiety-like behaviors and if anxiety-related phenotypes associated with deficiencies in one system can be compensated for by augmentation of the other.

Moreover, eCB signaling has been heavily implicated in the modulation of stress response physiology and subsequent behavioral responses used to model specific dimensions of mood and anxiety disorders. Studies have generally shown that stress evokes bidirectional changes in these two eCB molecules. For example, stress exposure reduces amygdala AEA levels, and reductions in AEA levels after stress correlate with anxiety-like behavioral states (16,31). In contrast to AEA, stress generally increases 2-AG levels (32), which contributes to termination and adaptation to stress, as well as potentially contributing to changes in pain perception and emotional memory (13,33).

Here we show that acute restraint stress causes a selective reduction in amygdala AEA levels and a robust anxiety-like behavioral state, which can be prevented by both AEA normalization and 2-AG augmentation. Moreover, acute 2-AG depletion causes an increase in anxiety-like behavior, which can be reversed by AEA augmentation. Physiological studies revealed amygdala glutamatergic transmission as a key predictor of anxiety-like state and anxiolytic response to 2-AG augmentation after stress. These studies support a functional reciprocal redundancy between two canonical eCB signaling systems in the modulation of anxiety-like behaviors.

METHODS AND MATERIALS

Subjects

Male ICR (CD-1) mice 6 to 9 weeks of age were used for all experiments (Envigo, Indianapolis, IN). All mice were group housed on a 12-hour light/dark cycle (lights on at 6:00 AM) with food and water available ad libitum. All studies were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (34) and were approved by the Vanderbilt University Institutional Animal Care and Use Committee (#M/15/105). All behavioral testing was performed between 6:00 AM and 6:00 PM.

Drugs and Treatment

The drugs used were the monoacylglycerol lipase (MAGL) inhibitor JZL184 (3, 5, 10, and 15 mg kg^{-1}), fatty acid amide hydrolase (FAAH) inhibitor PF-3845 (1 mg kg^{-1}), CB_1R agonist CP55940 (0.001, 0.01, 0.1, and 0.3 mg kg^{-1}), CB_1R agonist Δ^9-tetrahydrocannabinol (THC) (0.3 mg kg^{-1}), CB_1R antagonist rimonabant (2 mg kg^{-1}), and diacylglycerol lipase inhibitor DO34 (50 mg kg^{-1}). JZL184, PF-3845, rimonabant, or vehicle was administered by intraperitoneal (i.p.) injection at a volume of 1 mL kg^{-1} in dimethyl sulfoxide. DO34, CP55940, DO34+PF-3845, THC, or vehicle was administered by i.p. injection at a volume of 10 mL kg^{-1} in a formulation containing ethanol:Kolliphor:saline (1:1:18; Sigma-Aldrich, St. Louis, MO). Drug pretreatment times were 2 hours before behavioral testing except for THC (30 minutes). Diazepam was administered by i.p. injection 45 minutes before behavioral testing.

Supplementary Methods

For a detailed description of stress exposure, light/dark box test, lipid analysis, ex vivo slice electrophysiological recordings, and statistics, see Supplemental Methods and Materials.

RESULTS

MAGL Inhibition Reduces Anxiety-like Behavior

To elucidate the role for 2-AG signaling in the regulation of anxiety, we utilized the MAGL inhibitor JZL184. JZL184 administered at 3, 5, 10, and 15 mg kg^{-1} elevated 2-AG levels by 18%, 41%, 71%, and 135% (Figure 1A). In line with previous studies, elevations in brain 2-AG were accompanied by significant reductions in the levels of arachidonic acid and *N*-arachidonoylglycine (Figure 1B, C) (35). As expected, 2-oleoylglycerol levels were increased in a dose-dependent manner (Figure 1D). Importantly, brain AEA levels were unaffected by JZL184 at all tested doses (Figure 1E). Other *N*-acylethanolamines, such as *N*-oleoylethanolamine and palmitoylethanolamine, were likewise unaffected at any dose of JZL184 tested (Figure 1F [*N*-oleoylethanolamine] and Supplemental Figure S1A [palmitoylethanolamine]). Plasma 2-AG and 2-oleoylglycerol, but not AEA, were also increased in a dose-dependent manner by JZL184 treatment (Supplemental Figure S1B–F). JZL184 was detected at high levels in brain and plasma after i.p. injection (Figure 1G–H), and plasma JZL184 levels were positively correlated with brain JZL184 levels (Figure 1I) and 2-AG levels (Figure 1J), but not with AEA levels (Figure 1K). Plasma and brain 2-AG levels were also positively correlated after JZL184 treatment (Figure 1L).

We next sought to determine the efficacy of pharmacological MAGL inhibition on anxiety-like behavior using the light/dark box assay, a well-validated ethologically relevant measure of anxiety (36,37). Examination of time course data revealed JZL184 (3–15 mg kg^{-1}) increased light time, light distance, and total distance over time (Figure 1M–O). JZL184 also in a dose-dependent manner increased total percent light time and percent light distance, but not total distance traveled (Figure 1P–R). These data indicate that JZL184 has a small but significant anxiolytic effect under basal conditions. For comparative purposes, we examined the effects of diazepam and found as expected that it increased light time and light distance in this assay (Supplemental Figure S2).

MAGL Inhibition Prevents Stress-Induced Anxiety

As stress is the major environmental risk factor for the development of mood and anxiety disorders (38), we sought to determine whether increasing 2-AG–mediated CB_1 signaling could reduce stress-induced anxiety-like phenotypes. Mice tested in the light/dark box immediately after 30 minutes of restraint stress showed an anxiogenic response, which was prevented by diazepam treatment, supporting the validity and utility of the model (Supplemental Figure S3). Importantly, 30 minutes of restraint stress significantly reduced AEA and increased 2-AG levels compared with control mice in the amygdala, but not prefrontal cortex, and increased levels of corticosterone in both brain regions (Figure 2A–C). Moreover,