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2-Linoleoylglycerol Is a Partial Agonist of the Human Cannabinoid Type 1 Receptor that Can Suppress 2-Arachidonolyglycerol and Anandamide Activity

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Abstract

Introduction: The cannabinoid type 1 (CB1) receptor and cannabinoid type 2 (CB2) receptor are widely expressed in the body and anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are their best characterized endogenous ligands. The diacylglycerol lipases (diacylglycerol lipase alpha and diacylglycerol lipase beta) not only synthesize essentially all the 2-AG in the body but also generate other monoacylglycerols, including 2-linoleoylglycerol (2-LG). This lipid has been proposed to modulate endocannabinoid (eCB) signaling by protecting 2-AG from hydrolysis. However, more recently, 2-LG has been reported to be a CB1 antagonist.

Methods: The effect of 2-LG on the human CB1 receptor activity was evaluated *in vitro* using a cell-based reporter assay that couples CB1 receptor activation to the expression of the β-lactamase enzyme. Receptor activity can then be measured by a β-lactamase enzymatic assay.

Results: When benchmarked against 2-AG, AEA, and arachidonoyl-2′-chloroethylamide (a synthetic CB1 agonist), 2-LG functions as a partial agonist at the CB1 receptor. The 2-LG response was potentiated by JZL195, a drug that inhibits the hydrolysis of monoacylglycerols. The 2-LG response was also fully inhibited by the synthetic CB1 antagonist AM251 and by the natural plant derived antagonist cannabidiol. 2-LG did not potentiate, and only blunted, the activity of 2-AG and AEA.

Conclusions: These results support the hypothesis that 2-LG is a partial agonist at the human CB1 receptor and capable of modulating the activity of the established eCBs.

Keywords: 2-AG; 2-LG; ACEA; anandamide; cannabinoid receptors; endocannabinoid system

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Introduction

The cannabinoid type 1 (CB1) receptor and cannabinoid type 2 (CB2) receptor are widely expressed throughout the body with a plethora of functions.[1–3] For example, in the brain, CB1 receptors are expressed by embryonic and mature neurons, playing a role in axonal growth during development and in retrograde synaptic transmission at excitatory and inhibitory synapses in the adult.[4,5] CB1 and CB2 receptors are also expressed on neural stem cells and are involved in regulating several aspects of adult neurogenesis.[6–8] While inhibiting endocannabinoid (eCB) tone with CB1 antagonists has therapeutic potential for conditions such as obesity,[9] increasing tone with plant-derived cannabinoids and/or inhibitors of eCB hydrolysis has therapeutic potential for the treatment of many conditions that include multiple sclerosis and pain.[10,11]

Anandamide (AEA) was the first endogenous molecule to be identified with agonist activity at the cannabinoid receptors, with 2-arachidonoylglycerol (2-AG) identified as a second putative eCB soon after.[12,13] 2-AG is now regarded as the "workhorse" eCB based on experiments that show loss of all the major eCB responses in the brain when the enzymes responsible for the synthesis of 2-AG, diacylglycerol lipase alpha, and beta (DAGLα/DAGLβ) are knocked out.[7,14]

2-linoleoylglycerol (2-LG) is also synthesized by the DAGLs[15] and has previously been reported to potentiate

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2-AG activation of CB1 receptors in behavioral assays.[16] It was suggested that this might reflect an "entourage" effect with 2-LG limiting 2-AG breakdown by competing for binding to monoacylglycerol lipase (MAGL), the enzyme largely responsible for the hydrolysis of 2-AG.[17] However, experiments with cultured autaptic mouse hippocampal neurons show that 2-LG does not potentiate the effect of 2-AG on CB1-dependent depolarization-induced suppression of excitation (DSE). On the contrary, 2-LG displayed an antagonistic effect on the 2-AG response in this study.[18]

In this study, we evaluated the effect of 2-LG on the human CB1 receptor activity using a sensitive and quantitative cell-based reporter assay that couples receptor activation to the expression of the β-lactamase enzyme.[19] In this assay, the synthetic CB1 agonist arachidonoyl-2′-chloroethylamide (ACEA)[20] and the natural eCBs AEA and 2-AG serve as positive controls to benchmark 2-LG activity. The results clearly show that 2-LG exhibits the properties of a partial agonist at the CB1 receptor. Interestingly, the response was potentiated by JZL195, an inhibitor of the hydrolytic enzymes that limit eCB activity.[21] The 2-LG response was also fully inhibited by the competitive CB1 antagonist AM251[22] and by the noncompetitive allosteric antagonist cannabidiol (CBD).[23] 2-LG did not potentiate the effect of any concentration of 2-AG or AEA; in contrast, it substantially flattened the concentration–response curve of each of them. These results support the hypothesis that 2-LG is a partial agonist at the human CB1 receptor capable of modulating the activity of the established eCBs.

Materials and Methods

Materials

The Tango™ CNR1-bla U2OS cell line (Invitrogen, Life Technologies) was used in this study to measure CB1 receptor activation and has been extensively characterized by others.[19] In brief, it is based on the expression of a transgenic human CB1 receptor in the U2OS human osteosarcoma cell line. The CB1 receptor has a protease-sensitive transcription factor tagged at the C-terminus; the recruitment of a protease-tagged β-arrestin upon receptor activation releases the transcription factor which in turn stimulates expression of a β-lactamase reporter gene. Levels of β-lactamase are then detected in a standard enzyme activity assay using a fluorescence resonance energy transfer substrate.

ACEA and AM251 were purchased from Sigma Aldrich. 2-AG, 2-LG, and AEA were purchased from Cambridge Biosciences. CBD was purchased from Tocris. JZL195 was from Sigma-Aldrich. Compounds were reconstituted in dimethyl sulfoxide (DMSO) to a stock concentration and diluted in cell culture medium before use.

Cell culture

Tango CNR1-bla U2OS (CB1-Tango) cells were cultured as recommended by the supplier. In brief, they were grown in McCoy's 5A medium supplemented with 10% dialyzed fetal bovine serum, 0.1 M minimum essential medium nonessential amino acids, 25 mM HEPES buffer (pH 7.3), 1 mM sodium pyruvate, 100 μg/mL penicillin/streptomycin, 200 μg/mL Zeocin™, 50 μg/mL hygromycin, and 100 μg/mL geneticin, all from Invitrogen, Life Technologies. Cells were passaged according to manufacturer's instructions using typical aseptic culturing techniques and were incubated at 37°C in a humid atmosphere with 5% CO_2 content.

CB1-Tango assay

CB1-Tango cells were seeded in a 96-well black assay plate at a density of 30,000 cells per well in 100 μL of assay media (Freestyle 293 expression media from Invitrogen, Life Technologies) and incubated overnight in a 37°C, 5% CO_2 incubator. Compounds in DMSO were diluted in assay media to 6× concentrations, and 50 μL of total compound volume was added to appropriate wells (25 μL of agonist plus 25 μL of media, or 25 μL of agonist and 25 μL antagonist) and returned to the incubator for 4 h. Approximately 6× concentration of LiveBLAzer™-FRET B/G CCF4-AM Tango substrate (Invitrogen) was prepared according to manufacturer's instructions and 30 μL was added to each well. The plate was left in the dark for 90 min at room temperature and then read on the Flexstation microplate reader (Molecular Devices). Fluorescence emission was measured at 460 and 535 nm after excitation at 409 nm; CB1 activation is measured as the ratio of the signal at 460 and 535 nm, which was calculated for each well of the assay plate using SoftMax Pro software.

Data analysis

Data collected were analyzed using GraphPad Prism 5.0 or Microsoft Excel. Ratios obtained from CB1-Tango assays were averaged across technical repeats and normalized to control wells (control wells were either in the absence of any pharmacological drug or in the presence of AM251 only) as % of control in each

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experiment. Data were also normalized by subtracting the averaged control well ratio and normalized to the maximal eCB response (set to 100%) as % maximal eCB response. Normalized data were then averaged across independent experiments and plotted. Log EC_{50} values were obtained from nonlinear regression curve fitted to the data points generated by GraphPad Prism and averaged across experiments. All data are presented as mean ± standard error of the mean and were analyzed using Student's t-test (two groups) or one-way analysis of variance followed by Dunnett's post-test or Bonferroni Multiple Comparison post-test (more than two groups). Statistical significance between means was defined as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$.

Results

CB1-Tango cells detect CB1 activation by CB1 agonist and eCBs

Several cell reporter assays have been developed to characterize novel CB1 agonists and antagonists, including the CB1-Tango assay for the human CB1 receptor.[19] To be able to benchmark any potential 2-LG activity, we initially tested 2-AG, AEA, and the selective CB1 agonist ACEA in the assay. Representative concentration–response curves for each are shown in Figure 1. As expected, all three compounds stimulated robust responses within the case of ACEA (Fig. 1A), a statistically significant response obtained at 0.04 μM ACEA (185.5% ± 1.8% of control, $p < 0.001$) and a maximal response reached between 5 μM (259.2% ± 2.5% of control) and 25 μM (260.3% ± 1.3% of control). Detectable CB1 responses are seen at all tested concentrations of 2-AG (Fig. 1B) with a statistically significant response seen at 0.04 μM (140.6% ± 2.0% of control, $p < 0.001$) reaching a maximal between 5 μM (318.4% ± 9.0% of control) and 25 μM (325.5% ± 9.2% of control). AEA elicits a similar concentration-dependent response

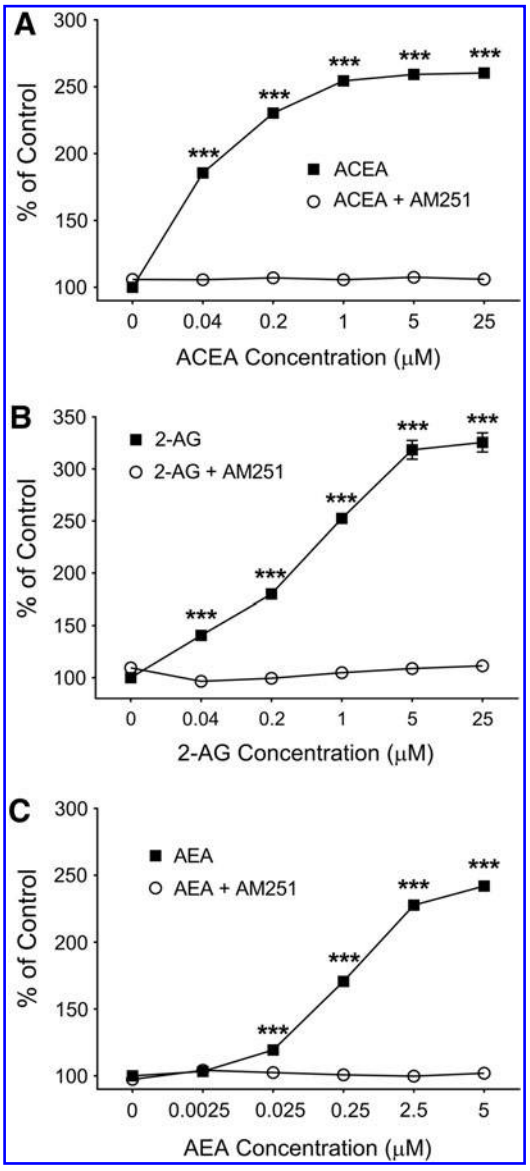


FIG. 1. CB1 agonist and eCBs stimulate CB1-dependent responses in CB1-Tango™ cells in a concentration-dependent manner. Concentration–response graphs of **(A)** ACEA, **(B)** 2-AG, and **(C)** AEA as agonists on the CB1-Tango cells in the absence and presence of CB1 antagonist AM251 (10 or 2.5 μM). Data are presented as mean ± SEM, from a single representative experiment. In these experiments, SEM that were below 2.5% may not be evident on the graph. Statistical significance of agonist responses compared to the control in the absence of agonist, and agonist responses in the presence of AM251 compared to AM251 only control, were established with one-way ANOVA, Dunnett's post-test. $^{***}p < 0.001$. ANOVA, analysis of variance; 2-AG, 2-arachidonoylglycerol; ACEA, arachidonoyl-2′-chloroethylamide; AEA, anandamide; CB1, cannabinoid type 1; eCB, endocannabinoid; SEM, standard error of the mean.

Table 1. Log EC_{50} Values Obtained of Cannabinoid Type 1 Agonists

Agonist	Log EC_{50}	*N*
ACEA	−7.735 ± 0.37	5
2-AG	−5.930 ± 0.42	4
AEA	−7.242 ± 0.35	6
2-LG	−4.781 ± 0.19	2–17

Log EC_{50} values of agonist concentration–response curves were calculated from each experiment and were pooled across experiments.
2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; ACEA, arachidonoyl-2′-chloroethylamide; AEA, anandamide.

with a significant response present at 0.025 μM (119.4% ± 2.4% of control, $p < 0.001$) and a maximal response between 2.5 (227.7% ± 1.8% of control) and 5 μM (242.0% ± 3.0% of control) (Fig. 1C). Importantly, none of the above compounds elicited a response when the selective competitive CB1 antagonist AM251 was present in the medium at 10 μM or at the lower concentration of 2.5 μM, which was used subsequently (Fig. 1A–C). AM251 on its own did not influence baseline CB1 activation, indicating that under the normal assay conditions, there is no basal eCB tone (data not shown). Based on a series of independent concentration–response curves, the EC_{50} values for ACEA, 2-AG and AEA were 0.018, 1.17, and 0.057 μM, respectively (Table 1).

2-LG shows partial agonism activity on CB1 receptor on the CB1-Tango cells

When tested in the CB1-Tango assay, 2-LG stimulated a significant CB1 response to 134.3% ± 4.18% of control value when used at 25 μM with no further increase at 50 μM (Fig. 2A). The CB1-Tango cells express eCB degrading enzymes MAGL, fatty acid amide hydrolase (FAAH), and ABHD6 (data not shown), and one or more of these might limit the activity of 2-LG based on their ability to hydrolyze monoacylglycerols. To test this, we repeated the experiments in the presence of 100 nM of JZL195—a potent and irreversible inhibitor of MAGL, FAAH, and ADBH6.[21] When added on its own, JZL195 stimulated a small response to 112.3% ± 3.65% of the control, with no response evident in the presence of 2.5 μM AM251 (98.5 ± 1.06%) ($n = 17$ independent experiments). This suggests that one or more of the above hydrolases are limiting the response to an endogenous ligand. In the presence of JZL195, 2-LG stimulated a more robust response at 12.5 μM, which peaked at 25–50 μM at around 170% of the JZL195 alone control response. This response was completely inhibited by AM251 (2.5 μM), confirming CB1 dependency. By way of comparison, Figure 2B shows the responses to the maximally active concentrations of 2-LG (25 μM),

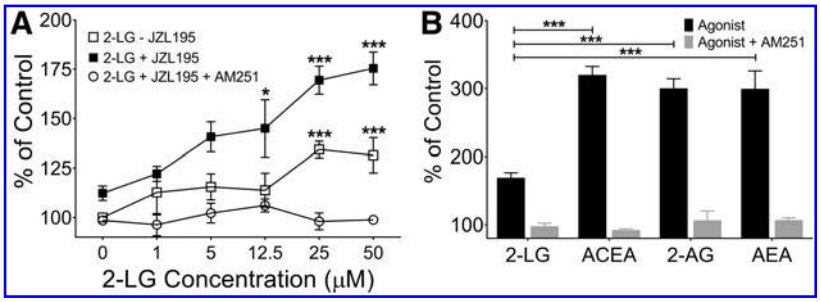


FIG. 2. 2-LG is a partial agonist of the CB1 receptor. **(A)** Concentration–response graph of 2-LG in the presence of 100 nM JZL195, 2-LG in the absence of JZL195, and 2-LG in the presence of both 100 nM JZL195 and 2.5 μM AM251. Data are presented as mean ± SEM of pooled independent experiments ($n = 2$–17 independent experiments) with eight replicates in each experiment. Statistical significance of 2-LG responses in the absence of JZL195 compared to its control, and 2-LG responses in the presence of JZL195 compared to JZL195 only control were established with one-way ANOVA and Dunnett's post-test. **(B)** Comparison of the maximal responses of 2LG (25 μM), ACEA (5 μM), 2-AG (25 μM), and AEA (5 μM) in the presence and absence of 2.5 μM AM251. One hundred nanomolar JZL195 was included only in 2-LG wells and experiments. Data are presented as mean ± SEM of pooled independent experiments (2-LG $n = 17$; ACEA $n = 13$; 2-AG $n = 4$; and ACEA $n = 7$) with eight replicates in each experiment. Significance between groups was established with one-way ANOVA and Bonferroni's multiple comparison post-test. ***$p < 0.001$. 2-LG, 2-linoleoylglycerol.

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ACEA (5 μM), 2-AG (25 μM), and AEA (5 μM) in the absence and presence of AM251 (2.5 μM) from several independent experiments and Table 1 compares the EC_{50} values. 2-LG clearly displays the properties of a relatively low-affinity partial agonist at the CB1 receptor.

CBD shows antagonistic activity against 2-LG and eCBs

When used at up to 5 μM, CBD has been characterized as a noncompetitive allosteric CB1 antagonist.[23,24] We have determined the effects of a similar range of CBD concentrations on the maximal responses to 2-LG (25 μM in presence of JZL195), ACEA (5 μM), AEA (5 μM), and 2-AG (5 μM), with the results pooled from several independent experiments shown in Figure 3.

In this set of experiments, 2-LG on its own elicited a response of 145.2% ± 3.1% of control. The 2-LG response is clearly inhibited by CBD with a small, but significant, inhibition at 0.2 μM (138.5% ± 6.3% of control) (Fig. 3A). In the presence of 5 μM CBD, the 2-LG response was not significantly different from the control value (104.5% ± 2.8%). In the case of the ACEA response, CBD had little effect when tested at up to 1 μM, but substantially inhibited the response by ~70% when used at 5 μM (Fig. 3B). Likewise, the response to AEA was also substantially inhibited (by ~72%) by 5 μM CBD (Fig. 3C). Surprisingly, the response to 2-AG was less sensitive to CBD with an ~34% inhibition seen at 5 μM. Nonetheless, others have also reported only a partial inhibition of the 2-AG response in a similar assay at this concentration of 2-AG and CBD.[23]

Collectively, these data show that when used at 5 μM, CBD fully antagonizes the 2-LG response and substantially (~70%) inhibits the ACEA and AEA responses, further supporting the hypothesis that 2-LG is a relatively low-affinity partial agonist at the CB1 receptor.

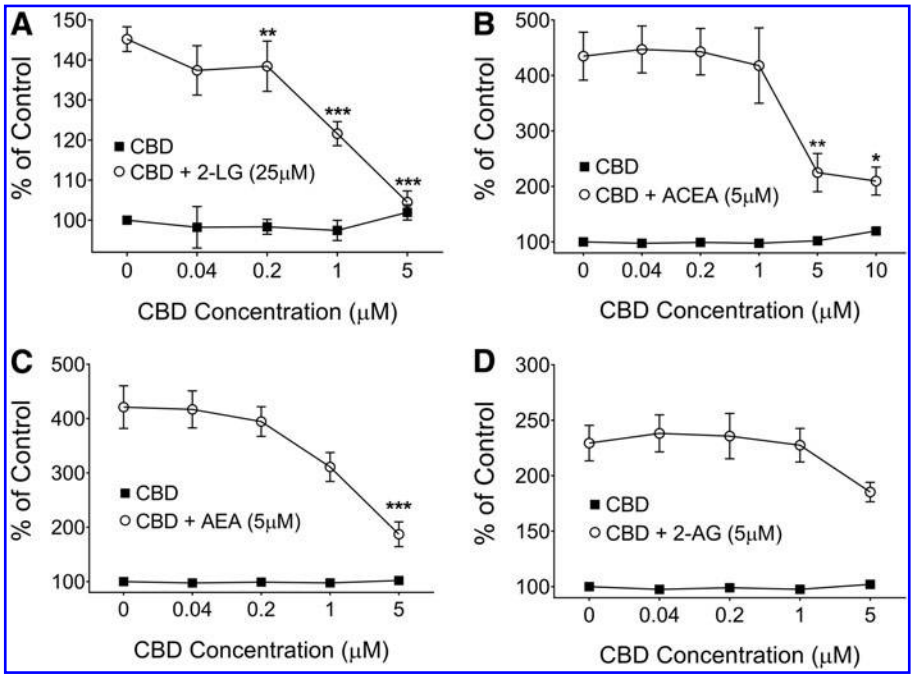


FIG. 3. CB1 agonist-elicited responses are antagonized by CBD. CBD response graphs in the absence and presence of CB1 agonists: **(A)** 25 μM 2-LG, **(B)** 5 μM ACEA, **(C)** 5 μM AEA, and **(D)** 5 μM 2-AG. Data are presented as mean ± SEM of pooled independent experiments (2-LG $n = 6$; ACEA $n = 7$; 2-AG $n = 9$; and ACEA $n = 8$) with eight replicates in each experiment. Statistical significance of agonist responses in the presence of increasing CBD compared to agonist alone were established with one-way ANOVA and Dunnett's post-test. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. CBD, cannabidiol.

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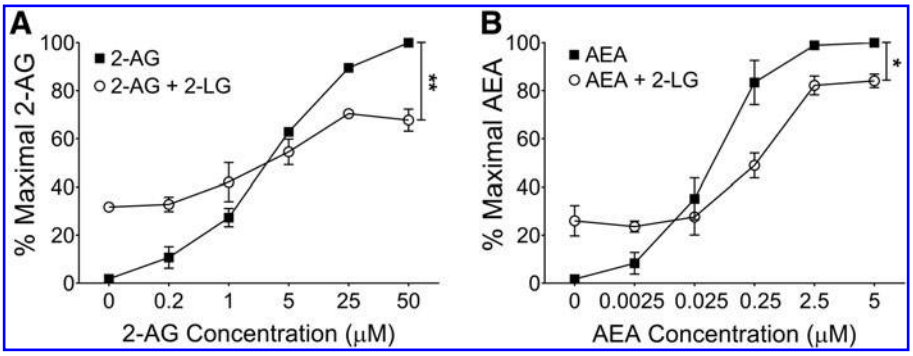


FIG. 4. 2-LG modulates CB1 activation by eCBs. Concentration–response curves of **(A)** 2-AG and **(B)** AEA in the absence and presence of 20 μM 2-LG. These experiments were conducted in the absence of JZL195. Data are presented as mean ± SEM of two independent experiments, six replicates from each experiment. Significance between the responses at the highest concentrations was established with unpaired Student's *t*-test, two tailed. *$p < 0.05$; **$p < 0.01$.

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2-LG can modulate CB1 activation by eCBs 2-AG and AEA

To further test if 2-LG acts like a partial agonist at the CB1 receptor, we evaluated its effect on 2-AG and AEA responses. For reasons that will be addressed in the discussion, these experiments were done in the absence of JZL195. Figure 4A shows the full dose–response curve to 2-AG conducted in the presence and absence of 20 μM 2-LG. An eCB tone of ~30% of the response of the full agonist has been generated by 2-LG on its own. The results also clearly show that the dose–response curve to 2-AG has been "flattened" by 2-LG. More specifically, there was no indication that the response to low doses of 2-AG were potentiated by 2-LG, nor are 2-AG and 2-LG responses ever additive. Indeed, it is apparent that the response to all active concentrations of 2-AG is suppressed by 2-LG. A very similar picture is seen with the dose–response curve to AEA; again, there is no evidence for 2-LG potentiating the response to low levels of AEA or for the 2-LG and AEA responses to be additive at any concentration. As with 2-AG, the response to active concentration of AEA is muted in the presence of 2-LG.

Discussion

The importance of the eCB system for the regulation of many complex behaviors, including appetite, pain, and anxiety, is well documented.[2,3,9] For example, gene deletion studies have shown that DAGLα is required for CB1-dependent DSE throughout the nervous system[7,14] and that DAGLα and DAGLβ are required for CB1-/CB2-dependent adult neurogenesis.[5,6,25] These studies point to 2-AG as the "workhorse" eCB in the brain regulating both synaptic and cellular plasticity. However, in addition to 2-AG, the DAGLs can synthesize other monoacylglycerols, including 2-LG.[15] This led us to consider if 2-LG might have a direct function at the cannabinoid receptors.

Conflicting reports have emerged regarding 2-LG effects on CB1 receptors. It was previously reported that 2-LG is unable to directly activate cannabinoid receptors, but instead can potentiate their activation by 2-AG in behavioral assays.[16,26] The mechanism for this potentiation is not clear, but it was suggested that 2-LG may limit 2-AG hydrolysis by competing at the substrate binding site on MAGL.[17] However, rather than enhancing 2-AG-/CB1-dependent synaptic plasticity in cultures of hippocampal neurons, 2-LG (at 5 μM) was reported by Murataeva et al. to inhibit the response.[18] Although elegant and of physiological relevance, the complexity of these models makes it difficult to dissect out the precise molecular basis of the action of 2-LG.

Over recent years, several CB1 reporter cell lines been developed to identify new receptor ligands. The Tango cell platform has been developed based on the introduction of key reporter transgenes into the human U2OS sarcoma cell line (see section "Materials and Methods" for details) and utilizes the physiologically important recruitment of β-arrestin to activated human CB1 receptors as the basis of the assay.[19,27] We have used this line to better understand the pharmacological properties of 2-LG at the human CB1

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receptor. To benchmark the activity of 2-LG, we first determined the effects of the well-characterized CB1 agonists ACEA, 2-AG, and AEA in the assay. They all stimulated robust responses that did not appreciably differ in magnitude, and the EC_{50} values for each were all within the published values obtained from [^{35}S]GTPγS binding assays.[20,28,29]

2-LG stimulated a response in the assay, but this was only robustly seen at $\sim$25–50 μM, and was highly variable. Transcriptional profiling detected MAGL, FAAH, and ABH6 in the reporter cell line (Doherty Laboratory unpublished results) and these enzymes can hydrolyze and thereby limit the activity of 2-AG and/or AEA in other cells.[21,30] When applied on its own, JZL195 stimulated a small, but significant, CB1-dependent response that most likely represents the unmasking of low-level eCB synthesis. This points to a "gate-keeper" function for one or more of the hydrolases in the U2OS sarcoma cells and interestingly, MAGL has a similar "gate-keeper" role in neurons where its exclusion from the growth cone facilitates 2-AG autocrine signaling to promote CB1-dependent axonal growth.[31] The response to 2-LG was much more robust and doubled in magnitude in the presence of JZL195, suggesting that hydrolysis is limiting its activity and perhaps explains, in part, the lack of agonist activity in other assays. Interestingly, studies on the genetic deletion of MAGL, FAAH or ABHD12 point to MAGL being the primary hydrolytic enzyme regulating 2-LG levels.[32,33] The possibility that the 2-LG response reflects an enhancement of the activity of an established eCB based on competition for binding to a hydrolase enzyme, along the lines of a previously postulated entourage effect, can be discounted as activity is seen in the presence of JZL195.

It is important to note that the 2-LG response was not seen when the selective CB1 antagonist AM251 was present. We were keen to confirm this with an independent antagonist. Hemopressin is a peptide that has been reported to be an inverse agonist at the CB1 receptor[34]; however, when tested at up to 20 μM, we failed to detect any effect on the response to low or high concentrations of ACEA, AEA, 2-AG, or 2-LG (Doherty laboratory unpublished observations). Others have also failed to find evidence for hemopressin activity at the CB1 receptor.[35–37] We therefore turned our attention to the natural plant-derived CBD. CBD has a low affinity for the CB1 receptor and functions as a noncompetitive allosteric antagonist.[23,38,39] When used at up to 5 μM, CBD fully inhibited the 2-LG response and substantially inhibited ($\sim$70%) the ACEA and AEA responses. However, its effect on 2-AG at this concentration was less pronounced, but others have also showed that it does not fully inhibit maximal 2-AG responses at this concentration in a similar β-arrestin recruitment assay.[23] Nonetheless, the key result here was the full inhibition of the 2-LG response as this provides independent evidence that it is a CB1-dependent response.

To further test if 2-LG is functioning as a partial agonist at the CB1 receptor, we tested it together with 2-AG and AEA. Importantly, when competing with a full agonist at the same receptor, a partial agonist will act like an antagonist. We conducted these experiments in the absence of JZL195 as we did not want to mask any potential entourage property that 2-LG might display toward 2-AG or AEA. In these experiments, on its own, 2-LG created an eCB tone that was around 25–30% of the 2-AG/AEA responses. The first important observation is that low concentrations of 2-AG/AEA that stimulate responses on their own fail to increase the tone in the presence of 2-LG—thus, we see no evidence for an entourage effect. Indeed, the opposite is clearly the case with all 2-AG/AEA responses being suppressed in the presence of 2-LG. Thus, 2-LG again exhibits the properties of a partial agonist.

The above experiments speak to the pharmacological properties of 2-LG and do not provide evidence for a physiological function. In general, 2-LG is present in the mammalian brain at around 10$\times$lower concentration than 2-AG, but perhaps 20$\times$higher than AEA.[40,41] However, 2-LG concentrations are higher in the hypothalamus,[40] and in some tissues, greatly exceed the level of 2-AG. For example, 2-LG is around 12$\times$higher than 2-AG in the spleen.[16] Thus, it is not unreasonable to suggest that in some instances, 2-LG might play a role in modulating 2-AG and/or AEA responses in a physiological context.

Pharmacological manipulation of the eCB system has been shown to have therapeutic benefits.[42,43] However, pharmacological intervention is not without risk as has been demonstrated by the adverse psychiatric side effects of Rimonabant, a synthetic CB1 antagonist that was developed to treat obesity,[44,45] and the very serious adverse psychotic effects of the highly potent synthetic cannabinoid agonists colloquially termed "Spice".[46] It is perhaps worth noting that some of the most promising medicines targeting eCB signaling are based on using the natural cannabinoids from plants, including Δ9-tetrahydrocannabinol and/or CBD,[47,48] or based on small molecule drugs that increase the levels of AEA[49,50]

or 2-AG.[51,52] In this context, strategies that increase 2-LG levels could have therapeutic potential as in some instances, they could introduce or maintain a moderate tone and/or mitigate against overactivation of the system.

Finally, while we have focused on the role of 2-LG at the CB1 receptor, additional roles at the CB2 receptor are likely and perhaps other receptors should be considered much like the case of AEA, which was considered a partial agonist of the CB1 receptor and a full agonist at the CB2 receptor, and subsequently shown to an active ligand at other receptors, including TRPV1.[51,52]

In conclusion, this study provides evidence to support the hypothesis that 2-LG is a partial agonist at the human CB1 receptor, is antagonized by CB1 antagonists AM251 and CBD, and can modulate the activity of the established eCBs *in vitro*.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

2-AG = 2-arachidonoylglycerol
2-LG = 2-linoleoylglycerol
ACEA = arachidonoyl-2′-chloroethylamide
AEA = anandamide
ANOVA = analysis of variance
CB1 = cannabinoid type 1
CB2 = cannabinoid type 2
CBD = cannabidiol
DAGLα = diacylglycerol lipase alpha
DAGLβ = diacylglycerol lipase beta
DMSO = dimethyl sulfoxide
DSE = depolarization-induced suppression of excitation
eCB = endocannabinoid
FAAH = fatty acid amide hydrolase
MAGL = monoacylglycerol lipase
SEM = standard error of the mean

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