ACCELERATED COMMUNICATION

Monoacylglycerol Lipase Activity Is a Critical Modulator of the Tone and Integrity of the Endocannabinoid System

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ABSTRACT

Endocannabinoids are lipid molecules that serve as natural ligands for the cannabinoid receptors CB1 and CB2. They modulate a diverse set of physiological processes such as pain, cognition, appetite, and emotional states, and their levels and functions are tightly regulated by enzymatic biosynthesis and degradation. 2-Arachidonoylglycerol (2-AG) is the most abundant endocannabinoid in the brain and is believed to be hydrolyzed primarily by the serine hydrolase monoacylglycerol lipase (MAGL). Although 2-AG binds and activates cannabinoid receptors in vitro, when administered in vivo, it induces only transient cannabimimetic effects as a result of its rapid catabolism. Here we show using a mouse model with a targeted disruption of the MAGL gene that MAGL is the major modulator of 2-AG hydrolysis in vivo. Mice lacking MAGL exhibit dramatically reduced 2-AG hydrolase activity and highly elevated 2-AG levels in the nervous system. A lack of MAGL activity and subsequent long-term elevation of 2-AG levels lead to desensitization of brain CB1 receptors with a significant reduction of cannabimimetic effects of CB1 agonists. Also consistent with CB1 desensitization, MAGL-deficient mice do not show alterations in neuropathic and inflammatory pain sensitivity. These findings provide the first genetic in vivo evidence that MAGL is the major regulator of 2-AG levels and signaling and reveal a pivotal role for 2-AG in modulating CB1 receptor sensitization and endocannabinoid tone.

Introduction

Endocannabinoids modulate a number of physiological processes, including pain, appetite, immune functions, and emotional state (Kaminski et al., 1992; Lee et al., 1995; Gallily et al., 2000; Klein et al., 2000; Di Marzo et al., 2001; Marsicano et al., 2002; Varvel and Lichtman, 2002; Hohmann et al., 2005; Klein and Cabral, 2006). They serve as natural ligands for the cannabinoid (CB) receptors and TRP channels. Endocannabinoid levels are tightly controlled by a balanced enzymatic biosynthesis and degradation in vivo. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are two central components of the endocannabinoid signaling networks (Cravatt et al., 2001; Ueda, 2002; Lambert and Fowler, 2005; Kogan and Mechoulam, 2006; Ahn et al., 2008). The hydrolysis of AEA is principally mediated by the membrane enzyme fatty acid amide hydrolase (FAAH) (Cravatt et al., 2001; Ueda, 2002; Klein and Cabral, 2006).

ABBREVIATIONS: CB, cannabinoid; 2-AG, 2-arachidonoylglycerol; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; CFA, complete Freund’s adjuvant; SNL, spinal nerve ligation; ANOVA, analysis of variance; CNS, central nervous system; ARA, arachidonic acid; LC/MS, liquid chromatography-tandem mass spectrometry; AA, arachidonic acid; SR141716, rimonabant; JZL184, 4-nitrophenyl [bis(1,3-benzodioxol-5-yl)[hydroxy]methyl]piperidine-1-carboxylate; WIN55212, (R)-(+-)[2,3-dihydro-5-methyl-3-(4-morpholinyl)methyl] pyrrolo-[1,2,3-d,e]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone; CP55940, 5-(1,1-dimethylheptyl)-2-(5-hydroxy-2-(3-hydroxypropyl)cyclohexyl)phenol.
Pharmacological inhibition of FAAH using selective inhibitors was shown to elevate AEA levels throughout the nervous system and periphery (Cravatt et al., 2001; Kathuria et al., 2003; Lichtman et al., 2004). FAAH-deficient mice lack AEA-hydrolyzing activity, and the endogenous brain levels of AEA are dramatically elevated in these animals, resulting in a variety of CB receptor-mediated behavioral phenotypes (Ahn et al., 2008). These results suggest that FAAH is responsible for AEA degradation in the brain.

Although several enzymes have been implicated in 2-AG hydrolysis, several lines of evidence suggest that the serine hydrolase monoacylglycerol lipase (MAGL) is the main contributor to 2-AG hydrolysis in vivo (Blankman et al., 2007; Kinsey et al., 2009). MAGL is abundant in brain tissues, in which it localizes to presynaptic terminals (Dinh et al., 2002). A comprehensive profile of brain serine hydrolases revealed that 85% of brain 2-AG hydrolase activity may be attributed to MAGL, with the newly identified enzymes ABHD6 and ABHD12 hydrolyzing the remaining 15% (Blankman et al., 2007). The newly identified MAGL-selective inhibitor 4-nitrophenyl 4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate (JZL184) was shown to increase the levels of brain 2-AG by 8-fold and reduce 2-AG hydrolysis by 85% (Long et al., 2009).

2-AG acts as a full agonist at both CB1 and CB2 receptors, whereas AEA was shown to act exclusively as a partial agonist at these receptors (Sugiura et al., 2002). Lack of many CB1-mediated behavioral effects in FAAH-deficient mice (Cravatt et al., 2001) suggests that CB1-mediated signaling may be predominantly mediated by 2-AG. Likewise, progenitor cell proliferation in the brain subventricular zone was shown to be reduced by both CB2 and 2-AG levels (Goncalves et al., 2008), suggesting that 2-AG is also a bona fide neurotrophic factor. The newly recognized role of endocannabinoids, particularly 2-AG, in adult brain neurogenesis underlies the pleiotropic nature of endocannabinoid functions and the therapeutic potential of these molecules in neurodegenerative diseases.

Here, we have tested the role of MAGL in controlling 2-AG levels in vivo by generating and characterizing a MAGL-deficient mouse. Lack of MAGL activity leads to an altered profile of endogenous 2-AG hydrolase activity and dramatic increase of 2-AG levels in the nervous system. Long-term elevation of 2-AG levels leads to desensitization of brain CB1 receptors, with a significant reduction of cannabimimetic effects of 2-AG and CB1 agonists. These findings provide the first genetic in vivo evidence that MAGL is the major regulator of 2-AG levels and signaling and reveal a pivotal role for 2-AG in modulating CB1 receptor sensitization and endocannabinoid tone.

Materials and Methods

**Generation of MAGL(-/-) Mice.** Approximately 13 kilobases of genomic DNA surrounding mouse MAGL exons 1 and 2 were cloned from a mouse BAC. Exons 1 and 2 were replaced by a loxP flanked PGK-neo cassette to allow for selection of homologous integration in C57BL6/Norc ES cells. The final targeting vector contained approximately 5.1 and 7.5 kilobases of MAGL genomic DNA on the 5’ and 3’ sides, respectively. Homologous integrants were identified by Southern blotting of KpnI-digested ES cell genomic DNA. All animals analyzed in this study lacked the selection cassette and were maintained on a C57BL6/Ntvac background.

**Preparation of Membrane-Bound MAGL.** Brains and other tissues were harvested from MAGL(+/+), MAGL(+/-), and MAGL(-/-) mice and immediately frozen on dry ice and kept at -80°C until use. The membrane-bound MAGL was prepared using high-speed centrifugation as described previously (Blankman et al., 2007).

**Recombinant MAGL Protein Expression.** The coding sequence of mouse MAGL was cloned into pCohIII expression vector (Takara-Mirus, Madison, WI) and expressed in Escherichia coli as described previously (Wang et al., 2008).

**MAGL Activity Assay.** MAGL activity was evaluated using its natural substrate 2-AG by incubation of recombinant MAGL protein or endogenous tissue extracts (20 μg) at room temperature followed by measurement of 2-AG hydrolysis products [arachidonic acid (AA)] by liquid chromatography-tandem mass spectrometry (LC/MS/MS) method as described previously (Zhang et al., 2010).

**Western Blot Analysis.** MAGL(+/-) or MAGL(-/-) mouse brain extracts were loaded onto a 4 to 12% SDS NuPAGE Bis-Tris polyacrylamide gel (Invitrogen, Carlsbad, CA) under reducing condition and was blotted onto a polyvinylidene difluoride membrane.

The membrane was probed using a rabbit polyclonal antibody (Cayman Chemical, Ann Arbor, MI) and the protein bands were visualized by enhanced chemiluminescence (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) as described previously (Wang et al., 2008). Measurement of 2-AG and AEA levels in the brains of MAGL(-/-) and MAGL(+/-) mice were quantified using a procedure described previously (Zhang et al., 2010).

**CB1 Receptor Binding Assay.** MAGL(+/-) and MAGL(-/-) mouse brains were dissected and homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.4, followed by centrifugation twice at 3800 g at 4°C. The resulting pellets were resuspended in 50 mM Tris-HCl, pH 7.4, 3 mM MgCl₂, and 1 mM EDTA. For in vitro binding assays, 72 μg of mouse brain protein was incubated at room temperature for 1 h with [3H]-[1-(1,1-dimethylheptyl)-2-(5-hydroxy-2-(3-hydroxypropyl)cyclohexyl)phenol (CP55940) in 0.05 ml of assay buffer (50 mM Tris-HCl, 5 mM MgCl₂, 2.5 mM EDTA, and 2.5 mg/ml bovine serum albumin) in the presence of 2 μM unlabeled CP55940. Reactions were terminated by rapid filtration using a 96-well filtration apparatus (Brandel Inc., Gaithersburg, MD) onto GF/B filter mats followed by washing (six times) with nonradioactive assay buffer without MgCl₂. Filter circles were placed in 7 ml scintillation vials with 3 ml of Opti-Fluor (PerkinElmer Life and Analytical Sciences, Waltham, MA), and the vials were counted on a PerkinElmer scintillation counter. Statistical significance was determined using Student’s t test.

**Behavioral Studies.** For the hot plate, mice (n = 8–10/group) were placed on a metal plate maintained at 52°C (Columbus Instruments, Columbus, OH). The latency to thermal nociceptive response (hind paw lift, flutter, licking, or escape behavior) was measured. Cutoff was set to 30 s. For the tail-flick, mice (n = 4–5/group) were placed on the Tail-Flick Unit (Ugo Basile, Varese, Italy), and an infrared beam was focused onto the tail, 5 cm from the tip. The latency for the animal to withdraw its tail was measured. Cutoff was set at 20 s. For the rotarod, mice (n = 4–5 group) were placed on the Tail-Flick Unit (Ugo Basile, Varese, Italy), and an infrared beam was focused onto the tail, 5 cm from the tip. The latency for the animal to withdraw its tail was measured. Cutoff was set at 20 s, and the intensity was set at 35% (R)(+)-(2,2-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo-[1,2-d]-1,4-benzoxazine-6-yl)-1-1-naphthalenyl-methanone (WIN55212) was administered intraperitoneally as a solution in 2% Tween 80/0.5% methyl cellulose. Latencies were determined at 1, 3, and 5 h after drug administration. Catalepsy was evaluated using the bar test, in which the animal’s front paws were placed over a thin horizontal rod 1.75 inches high (n = 8–10 /group). The amount of time (in seconds) for which the animal remains motionless was recorded (60 s maximum). Locomotor activity was assessed by placing each mouse separately in a 9 × 9-inch open field cage surrounded by clear Plexiglas walls (n = 8–10 /group). Infrared sensors placed outside the cage monitored the position of the animal in the cage. In the Rotarod Ataxia Assay, animals were placed on Rotarod (Ugo Basile), and the speed of Rotarod was set to accelerate from 4 to 40 rpm over a period of 300 s with the...
maximum time spent on the Rotarod set at 300s (n = 8–10/group). The amount of time spent on Rotarod was recorded, and data were presented as percentage of baseline latency. Thermal (hot plate) and mechanical (von Frey) hypersensitivity assessments in inflammatory (complete Freund’s adjuvant [CFA]) and neuropathic (spinal nerve ligation [SNL]) pain models (n = 8–10/group) were conducted as described previously (Whiteside et al., 2005). Statistical significance was determined using a one-way ANOVA followed by least squared differences post hoc analysis (SAS Institute, Cary, NC). The criterion for significant differences was set at p < 0.05. Male mice were used for behavioral experiments. Asterisks denote statistical significance compared with MAGL(+/+) controls.

Results

MAGL Hydrolyzes 2-AG and Maintains Its Levels In Vivo. The most abundant endocannabinoid in the brain, 2-AG, is primarily catabolized by a serine hydrolase MAGL that is believed to contribute to the maintenance of 2-AG levels (Ahn et al., 2008; Kinsey et al., 2009). An expression profiling study of mouse MAGL mRNA analysis reveals a relatively even distribution of MAGL expression across central and peripheral nervous system tissues (Supplemental Fig. S1).

We first analyzed 2-AG hydrolytic activity in brain extracts using the purified recombinant murine MAGL protein as control (Fig. 1A). Incubation with mouse brain extracts led to a rapid and time-dependent hydrolysis of 2-AG, with the concomitant appearance of its hydrolytic product arachidonic acid (Dinh et al., 2002) (Fig. 1A). More than 80% of 2-AG (50 μM) was hydrolyzed after 90 min in the presence of 20 μg of MAGL(+/-) brain extracts at room temperature (Fig. 1A).

To study the physiological role of MAGL in vivo, we generated mice lacking exons 1 and 2 of the MAGL genomic locus (Fig. 1B). MAGL(-/-) mice are viable and fertile and display the expected Mendelian frequency with no morphological defects. Lack of MAGL expression in the brain of MAGL(-/-) mice was confirmed by in situ hybridization and Western blot analyses (Fig. 1, C and D).

To determine whether deletion of MAGL resulted in compensatory changes in the expression of components of the endocannabinoid signaling pathway, we assessed the expression levels of FAAH, CB1, and CB2 receptors. No significant

Fig. 1. Characterization of the enzymatic activity of MAGL and targeted disruption of the MAGL gene in MAGL(-/-) mice. A, conversion of 2-AG into 1-AG or AA in the presence of recombinant MAGL protein or 20 μg of mouse membrane-enriched brain extract was compared in a 120-min time course. Data are presented as the mean percentage of 2-AG, 1-AG, and AA to the sum of all three ± S.E.M. B, schematic diagram of the targeting vector and the mouse MAGL genomic locus surrounding the deleted MAGL exons. C, in situ hybridization of brain hippocampus from MAGL(+/-) (+/+) and MAGL(-/-) (-/-) mice. D, Western blot analysis of recombinant MAGL protein (Ctrl) and three sets of brain lysates from MAGL(+/-) and MAGL(-/-) mice, respectively. Both mRNA and protein expression of MAGL are selectively absent in the MAGL(-/-) mice.
difference in mRNA levels of these genes could be observed between MAGL(+/-) and MAGL(-/-) mice (Supplemental Fig. S2).

A dramatic reduction in 2-AG hydrolysis was observed after incubation of 2-AG with MAGL(-/-) brain extracts compared with MAGL(+/-) (Fig. 2A) over the same time course. Only a small fraction (≈10-15%) of 2-AG was hydrolyzed in the presence of MAGL(-/-) brain extracts after 120 min (Fig. 2A). This residual 2-AG hydrolysis activity observed in MAGL(-/-) brain extracts may be attributed to the recently identified hydrolases ABHD6 and ABHD12 (Blankman et al., 2007). We used the previously described MAGL-selective inhibitor JZL184 (Long et al., 2009) to assess the residual 2-AG hydrolytic activity in MAGL-deficient mice. JZL184 significantly reduces 2-AG hydrolysis in MAGL(+/-) brain extracts but does not affect the reduced levels of 2-AG hydrolysis in the MAGL(-/-), further confirming the lack of MAGL activity in these mice (Fig. 2B). These data support the role of MAGL as the major 2-AG-hydrolyzing enzyme in the brain.

To further assess the role of MAGL in regulating endocannabinoid levels in vivo, we quantified the two major endocannabinoids, 2-AG and AEA, in brain, spinal cord, thymus, and spleen tissues (Fig. 2, C and D, and Supplemental Fig. S3) from MAGL(+/-) and MAGL(-/-) mice using LC/MS/MS (Zhang et al., 2010). Levels of 2-AG are dramatically elevated in MAGL(-/-) tissues compared with MAGL(+/-), with 30- and 16-fold increases in the brain and spinal cord, respectively (Fig. 2C). The levels of 2-AG were also increased in thymus and spleen (Supplemental Fig. S3). It is noteworthy that levels of AEA do not differ significantly between MAGL(+/-) and MAGL(-/-) mouse tissues (Fig. 2D). The dramatic and specific elevation of 2-AG levels in MAGL(-/-) mice confirms the pivotal role of MAGL as a regulator of 2-AG levels. The lack of change in AEA levels in MAGL(-/-) tissues compared with MAGL(+/-) controls further demonstrates that MAGL does not contribute to the maintenance of AEA levels.

2-AG-Mediated Behavioral Responses Are Not Dramatically Enhanced in MAGL(-/-) Mice. Adult male and female MAGL(-/-) mice exhibit an average decrease in total body weight of 16.5% compared with MAGL(+/-) controls at 3 months of age (Fig. 3A). Monitoring body weight over the first 6 weeks after birth reveals a persistent and statistically significant difference between MAGL(-/-) mice and MAGL(+/-) littermates (Fig. 3B). Surprisingly, the mutant mice do not display the typical behavioral features of cannabinoid receptor-mediated signaling despite a 30-fold increase in brain 2-AG levels compared with MAGL(+/-) (Fig. 3, C–G). No significant differences were observed in core body temperature (Fig. 3C), locomotion (Fig. 3D), and thermal nociception (Fig. 3, F and G) between MAGL(+/-) and mutant mice. MAGL-deficient mice displayed no significant ataxia (Fig. 3E) or catalepsy.

In the CFA model of peripheral inflammation, thermal and mechanical hypersensitivities developed at 24 h after CFA injection in MAGL(+/-) and MAGL(-/-) mice and were monitored for 2 weeks (Fig. 4A). MAGL(-/-) mice and MAGL(+/-) controls exhibit a comparable magnitude of thermal and mechanical hypersensitivity with a small but significant increase in thermal latency in the mutants only at 48 h post CFA (Fig. 4A). In the SNL model of neuropathic pain, no significant difference in the development of thermal and mechanical hypersensitivity could be observed between MAGL(-/-) and MAGL(+/-) controls (Fig. 4B). Our data indicate that elevated levels of 2-AG seen in MAGL(-/-) mice do not attenuate the development of inflammatory and neuropathic pain, in contrast with acute pharmacological inhibition of MAGL, which was shown to reduce pain hypersensitivity (Kinsey et al., 2009). Therefore, these findings suggest that cannabinoid signaling might be altered in the MAGL(-/-) mice, and this alteration can be evaluated by assessing exogenous and endogenous cannabinoid signaling in these mice.
CB1 Signaling Is Altered in MAGL(–/–) Mice. To assess whether the CB1 signaling pathway is still functional in the mutant mice, we looked at the effects of the prototypic CB1 agonist WIN55212 (Felder et al., 1995) in MAGL(–/–) and MAGL(+/+) mice. Administration of WIN55212 (10 mg/kg) led to an increase in thermal nociception threshold in MAGL(+/+) mice, as assessed in the hot plate and tail-flick assays. In the MAGL(–/–) mice, WIN55212 had no effect on the thermal threshold in the tail-flick assay, and a small but statistically significant effect was observed in the hot plate test (Fig. 5, A and B). Moreover, MAGL(+/+) and MAGL(–/–) mice exhibited significantly less catalepsy (20 to 40 s less than the MAGL(+/+)) at 1, 3, and 5 h after WIN55212 administration (Fig. 5C). These results indicate that CB1 receptor-mediated behavioral responses are altered in MAGL(–/–). This alteration in CB1 activity may be responsible for the unexpected lack of enhanced effects of 2-AG signaling in the mutant mice compared with MAGL(+/+) controls, despite the deficiency in 2-AG degradation in the mutants and subsequent elevation of 2-AG levels (Fig. 2).

The altered behavioral response to the CB1 agonist WIN55212 in MAGL(–/–) mice led us to assess the levels of CB1 receptor density and activity in samples from MAGL(+/+) and MAGL(–/–) brain. Ligand binding assay performed on brain membrane extracts from MAGL(+/+) and MAGL(–/–) mice (n = 5 per group) reveals a statistically significant 30% decrease in CB1 receptor density, but not ligand binding affinity, in MAGL(–/–) brain membrane extracts compared with MAGL(+/+), using the cannabinoid agonist [3H]CP55940 [B_{max} ± S.D.: MAGL(+/+) 2925.8 ± 529.9; MAGL(–/–) 2066.8 ± 393.6 fmol/mg] or antagonist rimonabant [3H]SR141716 [B_{max} ± S.D.: MAGL(+/+), 3668.0 ± 172.1; MAGL(–/–), 2647.3 ± 181.2 fmol/mg] (Supplemental Fig. S4). In contrast, CB2 receptor density levels are not significantly changed in the mutants as assessed by [3H]CP55940 binding on spleen tissue extracts from the MAGL(–/–) and MAGL(+/+) mice [B_{max}: MAGL(+/+), 601.8; MAGL(–/–), 549.2 fmol/mg].

To assess whether the decrease in membrane CB1 receptor density in the mutants results in a decrease in ligand-induced CB1 signaling, we measured guanosine 5′-3-O-(thio)triphosphate binding activity on brain membrane extracts from MAGL(–/–) and MAGL(+/+) mice, in the presence or absence of the agonist CP55940 (Supplemental Fig. S4). A statistically significant reduction in CP55940-mediated activation of CB1 signaling, as shown by a 2-fold increase in EC_{50} [MAGL(+/+), 4.4 ± 0.7; MAGL(–/–), 8.7 ± 0.9 nM] and approximately 25% decrease in E_{max} [MAGL(+/+), 81.8 ± 1.5; MAGL(–/–), 61.2 ± 0.9], was observed. Our data show that continuously enhanced 2-AG levels in the mutant mice lead to a reduction of CB1 receptor density and signaling in the brain.

Levels of 2-AG are tightly regulated by enzymatic biosynthesis and rapid degradation. Therefore, pharmacological studies to assess the physiological and behavioral implications of 2-AG administration in vivo are compromised by MAGL activity and 2-AG rapid degradation. In MAGL(–/–) mice, lack of MAGL activity leads to prolonged 2-AG half-life,
but altered CB1 signaling attenuates 2-AG effects. Probably as a result of the combination of increased 2-AG levels and decreased CB1 signaling, the physiological and behavioral effects of 2-AG administration in the mutant mice are overall comparable with those observed in the MAGL(+/+) controls, despite the lack of endogenous 2-AG degradation in the mutants (Supplemental Fig. S5).

Discussion

Endocannabinoids, including 2-AG, are produced on demand by neurons rather than being stored in synaptic vesicles like classic neurotransmitters (Di Marzo, 2008). The hydrophobic nature of these lipids enables them to freely diffuse across cell membranes and highlights the role of the degradative enzymes, including MAGL, as the major component of endocannabinoid signal termination. Biochemical and pharmacological studies suggest that 2-AG is abundant in the brain and that it is primarily hydrolyzed by MAGL (Dinh et al., 2002). The study of the full physiological role of 2-AG in vivo has been limited by the activity of endogenous 2-AG degrading enzymes, which quickly hydrolyze 2-AG and prevent it from signaling through the cannabinoid receptors. The recent characterization of JZL184 as a selective MAGL inhibitor (Kinsey et al., 2009; Long et al., 2009) provided the first tool to begin assessing the role of MAGL in vivo and elucidating of the physiological implications of 2-AG.

We provide here genetic evidence, using MAGL-deficient mice, that MAGL is the major 2-AG catabolic enzyme and a critical modulator of 2-AG levels in the CNS. MAGL-deficient mice with their high basal 2-AG levels, in the absence of significant endogenous degradation, allow the characterization of the role of this endocannabinoid in vivo. Our data demonstrate that 2-AG levels and signaling in vivo are primarily regulated by MAGL activity. MAGL(−/−) mice exhibit dramatically elevated endogenous brain and spinal cord levels of 2-AG (16- to 30-fold) with no significant alteration in AEA levels (Fig. 2, C and D).

Our data also provide in vivo evidence that long-term elevation in levels of endogenous 2-AG leads to down-regulation of CB1 signaling as assessed by the reduced response to CB1 agonist in MAGL(−/−) mice (Fig. 5). It is noteworthy that a decrease in brain CB1 but not spleen CB2 receptor density was observed, suggesting that only the brain CB1 receptor expression is altered in response to high 2-AG levels. The differential down-regulation between CB1 and CB2 receptors may be based on a distinction between CNS and peripheral tissues. The very low levels of CB2 expression in the CNS and the low levels of CB1 in peripheral tissues make quantifica-

![Fig. 4.](https://example.com/fig4.png)
tion of receptor activity and binding virtually impossible; therefore, we cannot exclude the possibility that levels of both receptors are altered in the CNS or that receptor levels are altered in the periphery.

MAGL (−/−) mice do not exhibit the classic tetrad of CB1-mediated behavioral and physiological effects, despite the dramatic increase in 2-AG levels. No significant effect on locomotor activity, Rotarod ataxia, and core body temperature was detected (Fig. 3, C–E). In addition, no difference in thermal nociceptive responses (hind paw lift, licking, or escape behavior) was observed in MAGL (+/+) and MAGL (−/−) mice (Fig. 3, F and G). Likewise, FAAH (−/−) mice do not exhibit the classic tetrad of CB1-mediated behaviors, despite large increases in anandamide levels. However, acute pharmacological blockade of MAGL produces significant hypothermic and hypomotility (Long et al., 2009), whereas no such effects are elicited by FAAH inhibitors. MAGL (−/−) mice do not exhibit a significant reduction in inflammatory or neuropathic pain hypersensitivity, despite the dramatic increase in brain 2-AG levels, suggesting that endocannabinoid signaling is altered in these mice. CB1 antagonism was also shown to modulate food intake and body weight (Di Marzo, 2009); therefore, the partial desensitization of CB1 receptor observed in MAGL (−/−) mice may be responsible for the apparent reduction in body weight in the mutant mice.

The combined effects of the lack of 2-AG hydrolytic activity and the decrease in CB1 signaling in MAGL (−/−) mice precluded a full interpretation of the behavioral effects of 2-AG administration in MAGL (−/−) mice. Unlike the response of FAAH-deficient mice to AEA administration (Cravatt et al., 2001), the decrease in CB1 signaling in MAGL (−/−) mice prevented the manifestation of dramatically enhanced 2-AG behavioral effects in these mice. The overall behavioral response to 2-AG administration in the mutant mice is the combined outcome of increased 2-AG half-life in the absence of MAGL activity and partial desensitization of CB1 receptors.

These results, together with the reduced response to the CB1 agonist WIN55212, suggest that 2-AG signaling is attenuated in the mutant mice through a down-regulation of CB1 receptor density and signaling in the brain. The small hypoalgesic response to WIN55212, observed in the mutants using the hot plate test at 1 h, could be the result of motor impairment, because there is an apparent small degree of catalepsy observed at the same time point. A recent study reported that upon administration of the selective MAGL inhibitor JZL184, brain levels of 2-AG increased by 8-fold, and JZL184-treated mice exhibited a wide array of CB1-dependent behavioral effects, including analgesia, hypomotility, and hypothermia (Kinsey et al., 2009). However, long-term administration of JZL184 may lead to an alteration in CB1 signalng similar to the alteration observed in MAGL-deficient mice. Taken together, our findings suggest that the CB1 receptor acts more as an “off switch” than a negative feedback loop in response to long-term elevation of 2-AG levels, leading to profound alterations in CB1-mediated functions in MAGL (−/−) mice. Modulation of brain CB1 and not spleen CB2 levels was observed in the mutant mice, suggesting that the CB1 signaling cascade is more sensitive to 2-AG levels or that brain CB1 and CB2 signalings are more sensitive to 2-AG levels than peripheral cannabinoid receptors.

In summary, we provide data showing that MAGL is a critical modulator of 2-AG levels and functions and that the endocannabinoid system adapts to long-term elevation of 2-AG levels by down-regulating CB1 receptor density and signaling. Moreover, our gain of function in vivo model of 2-AG signaling sheds light on the physiological and pathophysiological consequences of long-term inhibition of MAGL, a pharmacological target with therapeutic potential for neurologic and metabolic diseases.

**Fig. 5.** Cannabimimetic behavioral responses induced by CB1 agonist are diminished in MAGL (−/−) mice. CB1 agonist WIN55212 (10 mg/kg) (WIN) was administered intraperitoneally. Mice behavioral responses were recorded before WIN administration as baseline (BL). A, 1 h after administration, MAGL (+/+), MAGL (+/−), or MAGL (−/−) mice were placed on the tail-flick unit, and the latency for the animal to withdraw its tail after application of infrared beam was recorded. B, mice were placed on a hot (50°C) metal plate 1 h after WIN treatment, and the thermal nociceptive responses (hind paw lift, licking, or escape behavior) were measured. C, catalepsy was evaluated 1, 3, and 5 h after drug administration using the bar test. The amount of time for which the mice remains motionless was recorded. There was no catalepsy observed in mice from either genotype before drug application. The results are presented as mean ± S.E.M., 7–10 mice (20–30 g) per group. *, p < 0.05, **, p < 0.01 versus MAGL (+/+) mice, ANOVA.

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**References**


Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, and...