UC Irvine

UC Irvine Previously Published Works

Title

Assay of Monoacylglycerol Lipase Activity.

Permalink

<https://escholarship.org/uc/item/03g1h1fz>

Authors

Jung, Kwang-Mook
Piomelli, Daniele

Publication Date

2016

DOI

10.1007/978-1-4939-3539-0_17

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at

<https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Assay of Monoacylglycerol Lipase Activity

Kwang-Mook Jung and Daniele Piomelli

Abstract

Monoacylglycerol lipase (MGL) is a serine hydrolase involved in the biological deactivation of the endocannabinoid 2-arachidonoyl-*m*-glycerol (2-AG). 2-AG is one of the main endogenous lipid agonists for cannabinoid receptors in the brain and elsewhere in the body. In the central nervous system (CNS), MGL is localized to presynaptic nerve terminals of both excitatory and inhibitory synapses, where it helps control the regulatory actions of 2-AG on synaptic transmission and plasticity. In this chapter, we describe an *in vitro* method to assess MGL activity by liquid chromatography/mass spectrometry (LC/MS)-based quantitation of the reaction product. This method may be used to determine the basal or altered MGL activity in various cells or animal tissues after pharmacological, genetic, or biological manipulations. In addition, this assay can be used for MGL inhibitor screening using purified recombinant enzyme or MGL-overexpressing cells.

Key words Monoacylglycerol lipase (MGL), Enzyme assay, 2-Arachidonoyl-*m*-glycerol (2-AG), Arachidonic acid, Liquid chromatography/mass spectrometry (LC/MS)

1 Introduction

Monoacylglycerol lipase (MGL, also abbreviated MAGL or MGLL; EC 3.1.1.23, acylglycerol lipase), a member of the α/β -hydrolase domain (ABHD) family of serine hydrolases, catalyzes the conversion of long-chain monoglycerides (monoacylglycerols) into free fatty acids and glycerol (Fig. 1). The *MGLL* gene in humans encodes a 33 kDa protein composed of 303 amino acids (NCBI accession number: NP_001003794), which is responsible for the majority of MGL activity found in mammalian tissues [1, 2]. Other mammalian proteins contributing to this activity are ABHD6 and ABHD12 [3, 4].

MGL contains the GX SXG consensus motif common to most serine hydrolases, and harbors a catalytic triad composed of serine, aspartate, and histidine residues (Ser122-Asp239-His269 in human MGL) [1, 2]. Crystal structures (Protein Data Bank accession codes: 3HJU and 3JW8) indicate the presence of a canonical α/β -hydrolase fold characterized by a central β -sheet surrounded

Fig. 1 MGL catalyzes the conversion of monoacylglycerols into free fatty acids and glycerol. In this figure, 2-AG (monoarachidonoylglycerol) is converted to free arachidonic acid and glycerol by MGL. The catalytic serine residue in the MGL active site is shown in *red*

by six α -helices ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 6$, $\alpha 7$, and $\alpha 8$). In addition, $\alpha 4$, $\alpha 5$, and $\alpha 6$ form an U-shaped cap (or “lid”) domain that is likely to open upon interfacial activation allowing access of substrates to the enzyme’s active site [5, 6].

MGL is highly expressed in the central nervous system (CNS) [1, 2, 7], where it is the primary enzyme responsible for the hydrolytic degradation of the endocannabinoid, 2-arachidonoyl-*sn*-glycerol (2-AG), into free arachidonic acid and glycerol [8–11] (Fig. 1). Approximately 85 % of the 2-AG-hydrolyzing activity found in the rodent brain is attributable to this protein [1, 3, 12, 13]. MGL is also present in peripheral tissues where, in addition to degrading 2-AG, it completes the hydrolysis of triacylglycerols initiated by hormone-sensitive lipase and triacylglycerol lipase [14].

Earlier methods to measure MGL activity employed radioactive substrates such as 2- $[^3H]$ arachidonoylglycerol and thin-layer chromatographic separation, to assess radioactivity in breakdown products [1]. Advances in liquid chromatography/mass spectrometry (LC/MS) technology and greater availability of LC/MS instruments have contributed to the development of innovative methods for lipid analyses with greatly improved sensitivity, fidelity,

and accuracy [15, 16]. Here, we describe an LC/MS-based MGL assay that is routinely used in our laboratory [11, 13, 17]. The protocol includes details on enzyme preparation, lipid extraction, LC/MS analysis, and data processing.

2 Materials

Prepare all solutions using ultrapure water and analytical grade reagents. LC-grade solvents must be used for LC/MS analyses.

2.1 Reagents

2.1.1 Lipids

2-Oleoylglycerol (2-monoolein, 2-OG) (Sigma-Aldrich, St. Louis, MO, USA), heptadecanoic acid, and oleic acid (Nu-Chek Prep, MN, USA).

2.1.2 Buffers and Growth Medium

1. MGL homogenization buffer: 50 mM Tris-HCl, pH 8.0. Prepare 10× MGL homogenization buffer (0.5 M Tris-HCl, pH 8.0) and dilute to 1× when needed. For the 10× solution, weigh 60.57 g Tris and transfer to a glass graduated flask containing approximately 900 ml of water. Mix and adjust pH to 8.0 by adding HCl drop by drop. Add water to adjust the volume to 1 l. Store at 4 °C.
2. MGL reaction buffer: 50 mM Tris-HCl, pH 8.0, 0.05 % fatty acid-free bovine serum albumin (BSA). Weigh 25 mg of fatty acid-free BSA and dissolve them in 50 ml of 1× MGL Homogenization buffer. Store at 4 °C for 1–2 weeks.
3. 10× substrate solution: 0.1 mM 2-OG. Prepare just before use (*see Note 1*).
4. Stop solution: 100 % methanol containing internal standard (5 nmol heptadecanoic acid per sample). Prepare just before use (*see Note 2*).
5. Complete growth medium for HeLa cells: Dulbecco's modified Eagle medium plus 10 % fetal bovine serum and penicillin/streptomycin.

2.1.3 Solvents and Chemicals

1. Mobile phase A: Methanol, 0.25 % acetic acid, 5 mM ammonium acetate.
2. Mobile phase B: Water, 0.25 % acetic acid, 5 mM ammonium acetate.

2.2 Equipment

1. Tissue homogenizer.
2. Vortex mixer.
3. Water bath (37 °C).
4. Low-speed refrigerated centrifuge.
5. Spectrophotometer.

6. LC/MS system equivalent to or higher than Agilent 1200-LC system with autosampler linked to Ion Trap XCT or single-quadrupole 1946D MS detectors, and interfaced with ESI or APCI (Agilent Technologies, Wilmington, DE).
7. High-purity N₂ gas for sample drying.
8. Chemical fume hood (*see* **Note 3**).

2.3 Supplies and Apparatuses

1. Glass tubes (13×100 mm) or glass vials (8 ml) with Teflon liner cap.
2. Glass vials (1.5 ml) for LC/MS autosampler and conical inserts.
3. Glass pipettes and pipette aid.
4. (Micro)centrifuge tubes, micropipettes, and pipette tips.
5. Vial and tube racks.
6. 5' Glass Pasteur pipettes. Plug with sterile hydrophilic cotton.
7. LC column: Reverse-phase Zorbax XDB Eclipse C18 column (50×4.6 mm i.d., 1.8 μm, Agilent Technologies) or equivalent.

3 Methods

3.1 Enzyme Preparation

Comparative MGL assays can determine the basal or altered MGL activity in cells or in animal tissues under various conditions. Also, *in vitro* MGL activity assays can be performed to screen MGL inhibitors using MGL-overexpressing cells or purified recombinant enzyme (*see* **Note 4**). The following is a general protocol for enzyme preparation, which can be adapted by individual laboratories.

3.1.1 Cell Homogenate

1. Culture cells in appropriate tissue culture dishes, and treat them with test drug(s) if required.
2. On the day of harvesting cells, prepare an ice bucket, microcentrifuge tubes, glass tubes, and/or glass vials. Label tubes and vials appropriately.
3. Aspirate/discard the cultured media and wash cell cultures twice with a sufficient volume of ice-cold phosphate-buffered saline (PBS). Remove PBS completely.
4. Add ice-cold MGL homogenization buffer (1 ml per a 100 mm culture dish). Scrape and collect cells in (micro)centrifuge tubes.
5. Homogenize the cells using a tissue homogenizer or sonicator on ice. Settings may vary depending on the system (*see* **Note 5**). Be careful to prevent samples from heating up during homogenization.
6. Centrifuge samples for 10 min at 1000×*g* at 4 °C. Carefully collect the supernatant into clean tubes.

7. Determine protein concentration of the supernatant using the bicinchoninic acid (BCA) assay or Bradford protein assay, with BSA as a standard (*see Note 6*).

3.1.2 Tissue Homogenate

1. Collect the tissues of interest. If the tissues are going to be processed right away, keep them in cold MGL homogenization buffer on ice. Otherwise, snap-freeze them after sacrifice using liquid N₂ or powdered dry ice.
2. Place the (frozen) tissues into 10 tissue volumes of MGL homogenization buffer in an appropriate tube.
3. Homogenize the tissues using a tissue homogenizer or sonicator on ice. Be careful to prevent samples from heating up during homogenization. Settings may vary depending on the homogenization system and the amount and nature of tissue (*see Note 5*).
4. Centrifuge the samples for 10 min at 1000 × *g* at 4 °C. Carefully collect the supernatant into clean tubes.
5. Determine protein concentration of the supernatant by using the BCA assay or Bradford protein assay with BSA as a standard (*see Note 6*).

3.1.3 Homogenate of MGL-Overexpressing HeLa Cells

1. The day before transfection, detach HeLa cells from culture dishes by trypsin treatment and count them. Plate cells into 100 mm dishes using complete growth medium. The optimal number of cells to be plated varies depending on the transfection method.
2. Transfect the cells with plasmid DNA encoding MGL under a strong mammalian promoter. Our laboratory uses vectors encoding rat MGL under the human EF-1 α promoter [17].
3. Incubate the cells at 37 °C in a CO₂ incubator for 24–72 h after transfection.
4. Prepare cell homogenate according to Subheading 3.1.1.
5. Confirm MGL expression by Western blotting or other methods (*see Note 7*).

3.2 Setting Up the Enzyme Reaction

The final reaction consists of 50 mM Tris–HCl, pH 8.0, 0.05 % fatty acid-free BSA, an appropriate amount of enzyme, and 10 μ M 2-OG, in a total reaction volume of 0.5 ml. If required, MGL can be pre-incubated with test drugs for 10 min, alongside an appropriate vehicle. MGL activity is unaltered by dimethyl sulfoxide (DMSO) at concentrations ≤ 1 %.

1. Design the experiment. Determine the volume of enzyme source (χ μ l) that will be used for the assay, referring to the protein quantitation results (*see Note 8*). Run all reactions in triplicate. Include a blank triplicate assayed without any enzyme for blank subtraction.

2. Label glass tubes (13×100 mm) or glass vials (8 ml). Place them on ice.
3. Add (450− χ) μ l of MGL reaction buffer and χ μ l of enzyme source.
4. If testing a drug, add it to the mixture, vortex gently, and incubate in a 37 °C water bath for 10 min.
5. Quickly add 50 μ l of 10× substrate, vortex, and incubate at 37 °C for 30 min.

3.3 Lipid Extraction

1. Quench the reaction by adding 1 ml of Stop solution to each sample.
2. Add 2 ml of chloroform.
3. Vortex for about 60 s.
4. Add 0.5 ml of water.
5. Vortex for about 60 s.
6. Centrifuge the samples at 4 °C, 2000×*g* for 15 min. After centrifugation, the mixture should be separated into two phases with a whitish protein disk at the interface. The lower phase is mainly chloroform and contains most of the lipids; the upper phase is methanol and water containing more polar metabolites.
7. Prepare another set of 8 ml glass vials with the same labeling.
8. Collect the lower (organic) phase using a glass Pasteur pipette attached to a pipette aid (do not pipette by mouth!). Carefully transfer the organic phase to a new 8 ml glass vial (*see Note 9*). Discard the protein disk and the upper (aqueous) phase (*see Note 10*).
9. Evaporate the solvent to dryness in the vials with a gentle N₂ stream (*see Note 11*).
10. Resuspend the dried lipids in 0.1 ml of chloroform:methanol (1:3, vol/vol) mixture.
11. Transfer them into 1.5 ml glass LC vials with 0.2 ml conical inserts and proceed to LC/MS analysis.

3.4 LC/MS Analysis

Fatty acids are identified based on their retention times and MS properties.

1. Set up the column and the LC/MS parameters. We use a reverse-phase Zorbax XDB Eclipse C18 column (50×4.6 mm i.d., 1.8 μ m, Agilent Technologies). Detection and analysis are performed using Agilent Chemstation and Bruker Daltonics software.
2. Lipids are eluted with a linear gradient from 90 to 100 % of A in B for 2.5 min at a flow rate of 1.5 ml/min with the column

Fig. 2 Representative LC/MS chromatograms of oleic acid and heptadecanoic acid. Signals from LC/MS are extracted for oleic acid ($m/z=281$) and heptadecanoic acid ($m/z=269$). Insets show the peak integration summary report

temperature at 40 °C. ESI is in the negative mode, capillary voltage is set at 4 kV, and the fragmentor voltage is 100 V. N_2 is used as a drying gas at a flow rate of 13 l/min and a temperature of 350 °C. Nebulizer pressure is set at 60 psi. Fatty acids are measured by monitoring the mass-to-charge ratio (m/z) of deprotonated molecular ions $[M-H]^-$ in selected ion monitoring mode.

3. Extract chromatograms for oleic acid and heptadecanoic acid from LC/MS runs: For oleic acid, $m/z=281$, and for heptadecanoic acid, $m/z=269$. Figure 2 shows a representative LC/MS chromatogram, where the integrated peak area can be obtained using the LC/MS software.

3.5 Calculation of MGL Activity

The fatty acid product of MGL activity in this assay, oleic acid (18:1 Δ^9 FA), is quantified using the internal standardization method. Briefly, the method consists in adding known amounts of a structurally related internal standard to the sample under analysis. Unlike traditional analytical methods that rely on signal intensity, this method employs signal ratios.

In the current method, a fixed amount of standard heptadecanoic acid (17:0 FA) is added to the sample immediately following the incubation. Extracted chromatograms for oleic acid and heptadecanoic acid from LC/MS analyses are used to obtain the integrated peak area and the ratio of oleic acid to heptadecanoic acid for each sample. Then, the amount of oleic acid is determined based on a standard curve that is generated from mixtures of oleic acid and heptadecanoic acid with known ratios. This protocol is

very similar to the isotope dilution method that is commonly used to determine the quantity of endocannabinoids such as 2-AG and anandamide [15].

3.5.1 Standard Curve

1. Prepare 1 mM heptadecanoic acid and 1 mM oleic acid in a chloroform:methanol (1:3, vol/vol) mixture. Mix equal parts of 1 mM heptadecanoic acid and 1 mM oleic acid, to make the standard for 5 nmol oleic acid.
2. Dilute 1 mM oleic acid twofold with a chloroform:methanol (1:3, vol/vol) mixture to make 0.5 mM oleic acid solution. Mix equal parts of 1 mM heptadecanoic acid and 0.5 mM oleic acid, to make standard for 2.5 nmol oleic acid.
3. Dilute 0.5 mM oleic acid twofold with chloroform:methanol (1:3, vol/vol) mixture to make 0.25 mM oleic acid solution. Mix equal parts of 1 mM heptadecanoic acid and 0.25 mM oleic acid, to make standard for 1.25 nmol oleic acid.
4. Repeat twofold serial dilution of oleic acid and mix with 1 mM heptadecanoic acid to obtain (in nmol) 0.625, 0.3125, 0.15625, 0.078125, 0.0390625, and 0.01953125 standards.
5. Run each standard in duplicate by LC/MS. Generate a standard curve using the average peak area of the duplicates of each standard. The X-axis is the quantity (nmol) of oleic acid and the Y-axis is the ratio of oleic acid to heptadecanoic acid (Fig. 3) (see **Note 12**).

Fig. 3 A representative standard curve. An example standard curve for oleic acid quantitation using 5 pmol heptadecanoic acid as an internal standard is shown here. The result from a linear regression analysis of the data is also displayed. *Inset*: Magnification of the same graph in the range of 0–0.625 nmol oleic acid. 18:1 FA, oleic acid; 17:0 FA, heptadecanoic acid

3.5.2 Calculations

1. Using the peak area of samples obtained in Subheading 3.4, **step 3**, determine the ratio of oleic acid to heptadecanoic acid for each sample. Then, calculate the quantity of oleic acid by entering this ratio into the standard curve.
2. Calculate the quantity of oleic acid from the blank samples. Subtract the average of the blank samples from each sample run.
3. Normally, enzyme specific activity can be displayed as the amount of product formed per unit time and protein. In the current assay, MGL-specific activity is calculated as follows:

$$\text{specific activity (nmol/[min mg of protein])} = \frac{\text{oleic acid (nmol)} \times 1000 / \mu\text{g protein added to reaction} \times 30.}$$

4 Notes

1. First, prepare 10 mM 2-OG stock solution in DMSO and divide it into small aliquots (10–100 μl). Store at -20°C . Avoid repeated freeze-thaw. Just before use, dilute the aliquot 100 times with MGL reaction buffer (e.g., add 10 μl of 10 mM 2-OG to 990 μl MGL reaction buffer) and vortex to make 10 $\times$ substrate solution.
2. First, prepare 10 mM heptadecanoic acid stock solution in chloroform in a glass vial with Teflon liner cap. Protect from light and store at -20°C . Just before use, warm the 10 mM heptadecanoic acid stock solution at room temperature for about 5 min, and vortex. Prepare the Stop solution by adding the appropriate amount of 10 mM heptadecanoic acid stock solution to methanol, according to the number of samples to be analyzed (e.g., for ten samples, add 5 μl of 10 mM heptadecanoic acid stock solution in 10 ml of cold methanol). Keep on ice until use.
3. All procedures involving the use of chloroform should be handled in a chemical fume hood.
4. Our laboratory uses the pET15b vector system (Novagen, La Jolla, CA, USA) that produces rat MGL protein with an N-terminal 6x histidine tag. MGL is over-expressed in Rosetta 2(DE3)pLysS *E. coli* cells (Novagen), by using isopropyl- β -D-thiogalactopyranoside (IPTG) induction, and is purified from the 1 % Triton X-100-soluble fraction using a TALON column (Clontech, Mountain View, CA). For additional details, refer to our previous publication [13].
5. We use a Branson probe-type sonicator (Digital Sonifier 250, Branson Ultrasonics, Danbury, CT, USA) for cell homogenization, and a Polytron homogenizer (PT 6100, Kinematica AG, Luzern, Switzerland) for tissue homogenization. For example, we use 1-s pulse on-1-s pulse off, 10-s total running

Fig. 4 Dose-response curve for the MGL assay. MGL activity assay was performed as described in this chapter, using the indicated amounts of purified recombinant MGL. *Inset* shows the linear range of the dose-response relationship (0–10 ng purified MGL protein)

time for the Branson probe-type sonicator. Using the Polytron homogenizer, we homogenize tissues for 30 s to 1 min. All procedures should be done on ice and the samples should be kept cold at all times.

6. We determine protein concentration using a BCA assay kit (Life Technologies), and a spectrophotometer, following the manufacturer's manual.
7. We confirm MGL overexpression by Western blot using either anti-MGL antibody [1] or antibody for the C-terminal tag sequence of recombinant MGL [11]. Alternatively, quantitative PCR or MGL activity assay can be performed [11].
8. Determining the amount of protein being used for the assay is an important factor for success. As shown in Fig. 4, the increment of MGL enzyme in the assay shall eventually result in depletion of substrate, which causes the concentration of product to reach a plateau and saturate the assay system. Therefore, a comparative study must use protein amounts within the linear range of the dose-response relationship (1–10 ng for the purified MGL, inset of Fig. 4). Although the concentration of the 2-OG substrate vastly exceeds the concentration of enzyme under normal conditions, it is recommended to run an enzyme dose-response curve whenever a new type of protein source (cells, tissues, etc.) is used. In our laboratory, we use 0.2–5.0 μ g protein for HeLa MGL homogenate, 10–50 μ g for cell or brain homogenate, or 1–10 ng for purified recombinant MGL.

9. When the bottom organic phase is removed, it is important not to contaminate it with the upper aqueous phase. We suggest inserting the tip of the glass Pasteur pipette through the upper phase while giving a very gentle positive pressure (which will result in a gentle bubbling). When the tip has reached the bottom layer, carefully withdraw the organic phase from the very bottom of the glass tube. To prevent contamination, it is better not to try recovering the last drops from the bottom phase, instead leaving 5–10 % of the phase in the vial.
10. At this step, the procedure can be stopped. Keep the organic phase at -20°C .
11. (*Optional*) After **step 9**, the sample can be further purified by open-bed silica column purification if better separation is required for LC/MS analysis. Follow the **steps Opt 1 to Opt 9** below.
 - Opt 1. Reconstitute the dried lipid pellet from **step 9** in 2 ml of chloroform.
 - Opt 2. Prepare a suspension of Silica Gel G (60-Å 230–400 Mesh ASTM; Whatman, Clifton, NJ) in chloroform (1:1, vol/vol).
 - Opt 3. Add 1 ml of the Silica Gel G suspension to the glass columns.
 - Opt 4. Wash the columns with 2 ml of chloroform.
 - Opt 5. Load the samples onto the columns. Wait until all liquid drops down by gravity.
 - Opt 6. Elute the lipids with 2 ml of chloroform/methanol (9:1, vol/vol), and collect the eluate in another set of 8 ml glass vials with the same labeling.
 - Opt 7. Evaporate the eluate to dryness under a gentle N_2 stream.
 - Opt 8. Resuspend the dried lipids in 0.1 ml of chloroform:methanol (1:3, vol/vol) mixture.
 - Opt 9. Transfer them into 1.5 ml glass LC vials with 0.2 ml conical inserts, and proceed to LC/MS analysis.
12. The standard curve may be used to obtain oleic acid levels only when 5 nmol heptadecanoic acid is used as an internal standard. It is recommended to renew the standard curve periodically.

Acknowledgements

This work was supported by grants DA012413 and DA031387 from NIDA (to D.P.). The contribution of the Agilent Technologies/University of California, Irvine Analytical Discovery Facility, is gratefully acknowledged. We thank Dr. Collin Merrill for critical reading of the manuscript and Jennifer Daglian for technical assistance.

References

1. Dinh TP, Carpenter D, Leslie FM et al (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* 99:10819–10824
2. Karlsson M, Contreras JA, Hellman U et al (1997) cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem* 272:27218–27223
3. Blankman JL, Simon GM, Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 14:1347–1356
4. Marrs WR, Blankman JL, Horne EA et al (2010) The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* 13:951–957
5. Labar G, Bauvois C, Borel F et al (2010) Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. *Chembiochem* 11:218–227
6. Bertrand T, Augé F, Houtmann J et al (2010) Structural basis for human monoglyceride lipase inhibition. *J Mol Biol* 396:663–673
7. Gulyas AI, Cravatt BF, Bracey MH et al (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci* 20:441–458
8. Piomelli D (2003) The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 4:873–884
9. Chanda PK, Gao Y, Mark L et al (2010) Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system. *Mol Pharmacol* 78:996–1003
10. Schlosburg JE, Blankman JL, Long JZ et al (2010) Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* 13:1113–1119
11. Jung KM, Clapper JR, Fu J et al (2012) 2-Arachidonoylglycerol signaling in forebrain regulates systemic energy metabolism. *Cell Metab* 15:299–310
12. Dinh TP, Kathuria S, Piomelli D (2004) RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. *Mol Pharmacol* 66:1260–1264
13. King AR, Duranti A, Tontini A et al (2007) URB602 inhibits monoacylglycerol lipase and selectively blocks 2-arachidonoylglycerol degradation in intact brain slices. *Chem Biol* 14:1357–1365
14. Fredrikson G, Tornqvist H, Belfrange P (1986) Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim Biophys Acta* 876:288–293
15. Astarita G, Piomelli D (2009) Lipidomic analysis of endocannabinoid metabolism in biological samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 877:2755–2767
16. Piomelli D, Astarita G, Rapaka R (2007) A neuroscientist's guide to lipidomics. *Nat Rev Neurosci* 8:743–754
17. King AR, Dotsey EY, Lodola A et al (2009) Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chem Biol* 16:1045–1052