Regulation of GPR119 receptor activity with endocannabinoid-like lipids

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Gut hormones, such as glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), are peptides whose secretion from enteroendocrine intestinal cells is stimulated by the intake of nutrients. Released peptides signal to enhance nutrient metabolism and limit further nutrient consumption. These peptides play a crucial role in the regulation of glucose and lipid metabolism by stimulating insulin secretion, inhibiting glucagon secretion, improving insulin sensitivity, slowing gastric emptying, increasing satiety, and reducing food intake (12, 31).

Secretion of gut hormones is tightly regulated by the availability of nutrients (carbohydrates, amino acids, and lipids) in the intestine (13). A number of G protein-coupled receptors expressed in the enteroendocrine cells and activated by lipids have been shown to trigger secretion of GLP-1 and GIP (29). One of the most-studied of these receptors is known as GPR119 (7, 25). The Gα-coupled GPR119 receptor controls hormone secretion by boosting cellular cAMP levels upon activation (6, 7). The role of the GPR119 receptor in the regulation of gut hormone secretion and glucose homeostasis has been elucidated with the help of small molecule GPR119 agonists. GPR119 agonists exert glucose control by directly enhancing insulin secretion in pancreatic β-cells and by stimulating secretion of gut hormones (GLP-1, GIP, and PYY), which in turn induce additional increases in insulin secretion and improve hepatic glucose metabolism (6, 7, 15, 25). GPR119 agonists may therefore offer a good alternative to available type 2 diabetes treatment options by providing a stronger or safer glycemic control than achievable with those therapies.

GPR119 was initially described as a receptor for lysophosphatidylcholine (33). Subsequent work led to the identification of more potent and efficacious agonists for GPR119 belonging to the group of phospholipid-derived endogenous lipids known as endocannabinoid-like compounds. Endocannabinoid-like compounds are lipids that share with endocannabinoids their biosynthetic and degradation pathways but are devoid of cannabinoid receptor affinity (2, 23). The first such compound identified as a potent GPR119 agonist was N-oleylethanolamine (OEA) (25). OEA is synthesized in the proximal small intestine, where its concentration decreases during food deprivation and increases upon refeeding (16, 27). Additional endocannabinoid-like compounds, either amides of long-chain fatty acids or oleoyl-containing lipids, such as 2-oleoylglycerol (2-OG), were subsequently reported to increase GPR119 activity and serve as important endogenous or meal-associated agonists (8, 18, 19). With a large number of compounds that display different levels of GPR119 activation in vitro in the pharmacological assays, it is unclear which of these endogenous lipids actually mediates physiological activation of GPR119 in the intestine.

In this study, we systematically assessed the ability of an array of endogenous endocannabinoid-related lipids to activate GPR119 in vitro. We then determined the concentrations of several of the GPR119 ligands in the intestines of fasted and fed mice. Our data demonstrate that GPR119 is a promiscuous receptor that can be activated by a variety of endogenous endocannabinoid-like compounds, suggesting that some of these compounds could be involved in GPR119-mediated pathways.

MATERIALS AND METHODS

Materials. Compounds were obtained from Sigma-Aldrich (St. Louis, MO), Cayman Chemical (Ann Arbor, MI), Tocris Bioscience (Bristol, UK), or Enzo Life Sciences (Farmingdale, NY).

cAMP assays in cells expressing GPR119. Flp-In 293 T-REx cells (Life Technologies, Grand Island, NY) were used to generate stable cell lines expressing GPR119. The Flp-In T-Rex System combines the ability of the Flp-In System to make homogeneous cell lines using targeted integration with the inducible expression of the T-Rex System. Flp-In T-Rex-293 cells were cotransfected with the inducible expression vector pcdNAS/FRT/TO containing mouse GPR119 and the pOG44 vector encoding the Flp recombinase. A stable cell line was created using selection with 200 μg/ml hygromycin. For each

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assay, frozen cells expressing GPR119 were thawed, diluted with fresh media, and seeded in 96-well microplates. Cells were incubated at 37°C, 5% CO₂ for 3 h, and the media were removed. Compounds were serially diluted threefold in stimulation buffer [Earle’s balanced salt solution (EBSS), 2 mM glucose, 0.25 mM isobutyl methylxanthine, 0.1% BSA]. Cells were treated with compounds in the stimulation buffer for 60 min at 37°C, 5% CO₂. Accumulated cellular cAMP was then measured with a Tecan (Männedorf, Switzerland) Genios Pro instrument (excitation = 337 nM; emission = 650 nm/620 nM) using a homogeneous time-resolved fluorescence assay kit (CisBio, Bedford, MA). cAMP responses were normalized to the response produced by GPR119 agonist AR-231453 (6). CAMP elevation with 1 µM AR-231453 is defined as 100%.

GLP-1 secretion in GLUTag cells. GLP-1 secretion was determined in mouse endocrine GLUTag cells that express the proglucagon gene and secrete GLP-1 in a regulated manner (4). GLUTag cells were obtained from Dr. D. Drucker (University of Toronto). Cells were maintained at 37°C, 5% CO₂, 95% humidity in DMEM medium supplemented with 5.5 mM glucose, 10% FBS, and 2 mM glutamine. Before assay, cells were detached with Accutase (MP Biomedicals, Santa Ana, CA), pelleted, and seeded into 96-well tissue culture assay plates at the density of 15,000 cells/well. Cells were allowed to attach and then incubated for 72 h at 37°C, 5% CO₂. On the assay day, cells were washed two times with 200 µl EBSS buffer supplemented with 0.1% BSA. EBSS buffer (150 µl) containing a range of tested compound concentrations was added to cells, and plates were incubated at 37°C for 2 h. At the end of incubation, plates were spun down at 1,200 rpm for 1 min, and 100 µl of supernatant were collected and stored at −20°C. GLP-1 levels in the supernatant were determined with a Meso Scale Discovery (Gaithersburg, MD) total GLP-1 assay.

Animal studies. All in vivo studies were approved by Eli Lilly and Company’s Institutional Animal Care and Use Committee. Male C57BL/6 mice weighing 27–30 g were obtained from Harlan Laboratories (Indianapolis, IN) and were single housed on a 12:12-h light-dark cycle from 6:00 A.M. to 6:00 P.M. At 8:00 A.M. on the day before tissue collection, all animal cages were changed, and chow was removed. At 8:00 A.M. on the day of tissue collection, animals were randomized based on body weight. A subset of mice was fed for a period of 2 h, at which point animals in all groups were killed via CO₂ inhalation, and intestinal tissues were collected. Sections of the intestine were removed, washed in PBS, weighed, and snap-frozen at −80°C for storage until they could be used for lipid and RNA extraction. The following animal diets obtained from Harlan Laboratories were used in the studies: Teklad Global Protein Maintenance Diet (2014), AIG-93G basal mix (TD.00235), AIG-93G supplemented with 7% soybean oil (TD.99408), and AIG-93G supplemented with 7% lard oil (TD.120418).

Table 1. Stimulation GPR119 activity with lipids

<table>
<thead>
<tr>
<th>Compound</th>
<th>cAMP Response, %</th>
<th>Compound</th>
<th>cAMP Response, %</th>
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Results are mean cAMP responses from at least two observations. Each compound was tested at 10 µM in cells expressing GPR119. cAMP responses were normalized to the cAMP elevation induced with 1 µM AR-231453 (equal to 100%). OMDM-1, (S)-N-oleoyltyrosinol; OMDM-2, (R)-N-oleoyltyrosinol; HpEPE, hydroperoxyicosatetraenoic acid; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; GABA, γ-aminobutyric acid; DiHETE, dihydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid.
Liquid chromatography-mass spectrometry procedures. Pure deuterated synthetic standards of lipids were purchased from Cayman Chemical. Stock solutions of each lipid standard (100 µg/ml) were prepared by dissolving dry solids in acetonitrile. Extraction of lipids from intestinal samples was done with cold acetonitrile containing internal lipid standards. Acetonitrile was added to samples to produce 100 mg/ml concentration of the homogenate tissue. The tissue samples were disrupted with TissueLyser (QIAGEN), and samples were centrifuged. The resulting supernatant was transferred to 96-well plates, and acetonitrile was removed on a Minivap (Porvair Sciences). The residue was taken up in 100 µl of methanol-water-formic acid (80:20:0.4%) for liquid chromatography-mass spectrometry (LC-MS) analysis. LC/electrospray ionization (ESI)/MS/MS analysis of lipids was performed using a TSQ Quantum Ultra-Triple quadrupole mass spectrometer (ThermoFisher, San Jose, CA) equipped with an ESI probe and interfaced with the Agilent 1290 infinity LC system (Agilent, Palo Alto, CA). The HPLC system consisted of an Agilent 1100 binary pump and a CTC Leap Technologies HTS Pal Autosampler (Carrboro, NC) equipped with a 100-µl syringe, Valco C6WK injector (Houston, TX), and 10-µl sample loop. The lipid extracts were separated via elution with methanol-0.1% formic acid on an Eclipse Plus C18 RRHD, 2.1 × 50 mm, 1.8-µm column (Agilent). Mass spectrometric analyses were performed online using ESI tandem mass spectrometry in the positive multiple-reaction monitoring mode. Lipid levels were quantified by the ratio of analyte to internal standard, and calibration curves were obtained by serial dilution of a mixture of lipids.

RESULTS

We studied GPR119 receptor activation by measuring cAMP levels in cells expressing GPR119. A library of lipids that included either N-amides of long-chain fatty acids or unsaturated fatty acid conjugates with different polar heads was screened at a concentration of 10 µM. The small molecule GPR119 agonist AR-231453 (6) served as a positive control for the assay, in which it generated a fourfold increase in cellular cAMP levels with EC50 of 9 nM. Of the almost 50 compounds tested in the assay, two dozen showed receptor activation of 20% and higher (Table 1). We confirmed that the observed increases in cAMP levels were specific for cells expressing GPR119 by measuring effects of the most efficacious GPR119 agonists on cAMP levels in parental 293 cells that do not express GPR119 (data not shown). The potency and relative efficacy of the most active representative compounds of different structural classes were further defined by determining their concentration-response curves in the GPR119 cAMP assay.
thanolamine (POEA), and linoleylethanolamine (LEA) were particularly potent in activating the receptor (EC50 values of 5 \mu M for each lipid, Fig. 1A), whereas N-palmitoylethanolamine (PEA), N-dihomo-linolenylethanolamine, and N-stearoylethanolamine were less potent. Oleic, linoleic, and palmitoleic acids, which constitute the alkyl chains of OEA, POEA, and LEA, respectively, did not increase cAMP levels in GPR119 cells (data not shown).

Epoxycosatrienoic acid ethanolamines (EET-EA), which are cytochrome P-450-mediated oxygenation products of N-arachidonylethanolamine (AEA) (32), demonstrated considerably greater potency in activating GPR119 than did the parent AEA (Fig. 1B). The exact location of the epoxy group on the fatty acid chain did not affect the potency of EET-EAs in activating the receptor (EC50 values of all EET-EAs ca 14 \mu M). However, one of the EET-EAs, 14(15)-EET-EA, displayed <100% efficacy, whereas another lipid, 8(9)-EET-EA, was found to be more efficacious (120% relative efficacy) than the reference compound.

We also tested the ability of oleic acid conjugates bearing different polar groups to activate the receptor (Fig. 1C). 2-OG, 1-oleoyl-2-acetylglycerol, and 1-oleoyl lysophosphatidic acid proved less potent than OEA itself, and oleoyl serotonin behaved as a partial receptor agonist. For 2-OG, the GPR119 activation EC50 value was around 17 \mu M. Interestingly, 10 \mu M 2-linoleoylglycerol (2-LG) did not activate GPR119 (Table 1).

Fig. 2. Arvanil antagonizes GPR119 receptor activation. A: concentration-dependent inhibition of OEA-induced cAMP increase with arvanil in cells expressing GPR119. OEA (30 \mu M) was used to induce an increase in cAMP. B: concentration-dependent inhibition of AR-231452-induced cAMP increase with arvanil in cells expressing GPR119. AR-231452 (1 \mu M) was used to induce an increase in cAMP. Data are shown as means ± SE of three observations.

Fig. 3. GPR119 receptor agonists stimulate glucagon-like peptide-1 (GLP-1) secretion in GLUTag cells. AR-231453 was tested at 10 \mu M, and all other compounds were tested at 30 \mu M. Data are shown as means ± SE of at least four observations. *P < 0.05 and ***P < 0.001 vs. GLP-1 secretion of the vehicle group.

Fig. 4. Arvanil antagonizes GLP-1 secretion induced with GPR119 receptor agonists. A: concentration-dependent inhibition of AR-231453-induced GLP-1 secretion with arvanil. GLP-1 secretion was measured in GLUTag cells in the presence (●) or absence (◼) of AR-231453. B: 50 \mu M arvanil inhibits concentration-dependent increase in GLP-1 secretion induced by AR-231453. GLP-1 secretion was measured in GLUTag cells in the presence (●) or absence (◼) of 50 \mu M arvanil. Data are shown as means ± SE of eight observations.
We wondered if synthetic endocannabinoid analogs with an oleic acid chain, such as (S)-N-vanillyloleamide, (S)-N-oleoyltyrosinol, and (R)-N-oleoyltyrosinol, which were developed either as potent inhibitors of endocannabinoid cellular transport or as CB₁ agonists (3, 24), might also activate GPR119. These compounds were found to be fairly potent in activating the GPR119 receptor but acted as partial agonists, producing roughly one-half of the maximal receptor activation achieved with AR-231453 (Fig. 1D). Furthermore, they were less potent at activating GPR119 than they were at transient receptor potential vanilloid 1 (TRPV1) or at inhibiting the anandamide cellular reuptake.

We also examined whether endocannabinoid-like lipids might antagonize the OEA-induced cAMP elevation in cells expressing GPR119 receptor (data not shown). N-vanillylarachidonamide (arvanil), a synthetic endocannabinoid analog that can activate CB₁ receptors and TRPV1 channels as well as inhibit endocannabinoid cellular transport (10, 22), was found to be a GPR119 antagonist. Arvanil did not activate the GPR119 receptor on its own (Fig. 1D) but inhibited the increase in cAMP levels induced either by 30 μM OEA or 1 μM AR-231453 in a concentration-dependent manner (Fig. 2, A and B).

The GPR119 receptor has been shown to play an important role in the regulation of incretin hormone secretion from intestinal L cells (7). Therefore, we were also interested in studying the effects of these lipid GPR119 agonists on GLP-1 secretion in mouse enteroendocrine GLUTag cells that express the proglucagon gene and can secrete GLP-1 in a regulated manner (4). The ability of the lipids to stimulate GLP-1 secretion was tested in this cell line at 30 μM lipid concentration. AR-231453 was included as a positive control (Fig. 3). OEA, LEA, and POEA, the NAEs that showed the greatest potency in activating GPR119, produced the strongest stimulation of GLP-1 secretion in GLUTag cells (Fig. 3). Less potent

![Graph](http://www.ajpendo.org)

**Fig. 5.** OEA-stimulated GLP-1 secretion in GLUTag cells is blocked by GPR119 antagonist arvanil (50 μM) but not by transient receptor potential vanilloid 1 (TRPV1) antagonist SB-366791 (10 μM), cannabinoid receptor agonist WIN-55,212-2 (1 μM), peroxisome proliferator-activated receptor (PPAR) α/γ agonist tesaglitazar (20 μM), and PPARα/γ antagonist GW-9662 (5 μM). GLP-1 secretion was measured in the absence (open bars) and presence (filled bars) of 30 μM OEA. Data are shown as means ± SE of 14 observations. **P < 0.01 and ***P < 0.001 vs. GLP-1 secretion without OEA in the corresponding group.

![Graph](http://www.ajpendo.org)

**Fig. 6.** Intestinal levels of N-acyl ethanolamines (NAEs) in fasted (open bars) and refed (filled bars) mice. Mice were kept on 2014 diet, starved overnight, and then given access to food for 2 h. Levels of OEA (A), LEA (B), PEA (C), and AEA (D) were measured in different sections of intestine. Data are shown as means ± SE of 12 observations. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the corresponding lipid level at the fasted state.

**E1473 ENDOCANNABINOID ACTIVATION OF GPR119**
GPR119 agonists produced a slight but significant increase in GLP-1 secretion.

We also examined the effects of arvanil, the GPR119 receptor antagonist, on GLP-1 secretion in GLUTag cells. Arvanil did not significantly modulate GLP-1 secretion under basal conditions but produced concentration-dependent inhibition of AR-231453-induced GLP-1 secretion (Fig. 4A). Moreover, 50 µM arvanil fully blocked the concentration-dependent increase in GLP-1 secretion generated with AR-231453 in GLUTag cells (Fig. 4B).

NAEs are known to activate a number of molecular targets (2, 26). It is therefore possible that OEA-stimulated GLP-1 secretion proceeds via GPR119 receptor-independent pathways. To explore this possibility, we studied the effects of specific pharmacological modulators of receptors known to be activated by some NAEs on OEA-stimulated GLP-1 secretion in GLUTag cells. GLP-1 secretion in response to 30 µM OEA was measured in the presence of the GPR119 antagonist arvanil, the TRPV1 antagonist SB-366791 (17), the cannabinoid receptor agonist WIN-55,212–2 (14), the peroxisome proliferator-activated receptor (PPAR) α/γ agonist tesaglitazar (9), and the PPARα/γ antagonist GW-9662 (21) (Fig. 5). Only the GPR119 antagonist arvanil completely blocked the OEA-induced increase in GLP-1 secretion. WIN-55,212–2 partially inhibited OEA-induced GLP-1 secretion (Fig. 5), a result that can be rationalized by cannabinoid receptor activation counteracting the GPR119 agonist-induced rise in cAMP levels. The TRPV1 agonist SB-366791 lowered both basal and OEA-induced GLP-1 secretion to a similar extent, confirming previous observations on the role of TRPV1 in the regulation of GLP-1 secretion (34). The PPAR ligands tesaglitazar and GW-9662 had no impact on basal or OEA-induced GLP-1 secretion.

To assess the possibility that some of the identified ligands may act as endogenous GPR119 receptor agonists, we determined their distribution along the gastrointestinal tract in mice. Lean C57BL/6 mice were kept on a grain-based 14% protein chow diet (2014, Teklad Diets; Harlan Laboratories). The levels of NAEs were determined in different sections of the mouse intestine for animals in the fasted state as well as animals that had been fasted and then refed. In the fasted state, PEA was the most abundant NAE followed by LEA and OEA (Fig. 6, A, B, and C). AEA was the least abundant of all measured NAEs (Fig. 6D), and no 8(9)-EET-EA could be detected in any portion of the intestine under the study conditions (data not shown). We did not measure POEA levels, since it is known that this lipid is not found in the mouse intestine (16).

Upon refeeding with chow diet, PEA levels did not change, whereas LEA levels were robustly increased in all studied intestinal segments (Fig. 6, B and C). The greatest increase in LEA following feeding, 40-fold, was detected in the jejunum (Fig. 6B). In the refed state, LEA levels by far surpassed those of PEA, making LEA the most abundant NAE detected in any portion of the intestine. Refeeding resulted in a modest two- to fourfold increase in OAE levels in the proximal part of the intestine (duodenum and jejunum), whereas no significant changes in OEA levels were observed in the distal intestine (Fig. 6A). The only NAE whose levels were lower in fed than in fasted animals was AEA (Fig. 6D).

The intestinal samples used for lipid determination were also used to study mRNA expression of GPR119 as well as markers for enteroendocrine cells, GIP, proglucagon, and PYY (Fig. 7).
Short-term fasting/refeeding did not cause any significant changes in mRNA levels of any of the studied genes. In both nutritional states, GPR119 receptor expression was detected in all intestinal segments, with the highest GPR119 mRNA signal in the distal part of the intestine-colon followed by ileum (Fig. 7A). GIP mRNA, the marker of K cells, was detected in the proximal parts of intestine-duodenum and jejunum (Fig. 7B), whereas mRNA for proglucagon and PYY, L cell markers, were detected in ileum and colon (Fig. 7, C and D).

The grain-based 2014 diet used to study intestinal lipid levels contains more linoleic than oleic acid, 2 and 0.7%, respectively. To explore the role of the fatty acid composition of the diet in the regulation of endocannabinoid-like lipids in intestine, we used the purified AIN-93G diet that is composed of refined ingredients, including purified proteins, sugars, and vitamins (28). Three modifications of AIN-93G with different fat composition were used (no fat added, 7% soybean oil, and 7% lard oil). Use of the two oils allowed variation in fat composition. Linoleic acid is a major component of soybean oil (53%), whereas oleic acid is the prevalent fatty acid in lard oil (45%). Mice were kept on these diets for 1 wk before lipid levels were measured in jejunum segments from fasted and re-fed animals. The change from the natural 2014 diet to the purified AIG-93G diet produced dramatic decreases in intestinal levels of OEA and LEA in the fed state, whereas PEA and AEA levels were less affected (Fig. 8), confirming earlier findings that intestinal NAE levels decline in animals kept on the purified diets (1). Despite the drop in the absolute levels, increases in OAE and LEA levels at the fed state seen in mice on the 2014 diet were also observed in mice on the AIG-93G diets, although at much smaller magnitudes. LEA levels significantly increased in the fed state in mice receiving diets with soybean or lard oils, whereas no changes in LEA levels were observed in animals fed with the fat-free diet (Fig. 8B). OEA levels were slightly elevated only in mice receiving the lard oil diet (Fig. 8A). Even in animals fed with the diet containing oleic acid as major fat component, OEA levels were somewhat lower than LEA levels. As was the case for animals receiving the 2014 diet, PEA levels did not change between fed and fasted states (Fig. 8C), and AEA levels decreased in mice fed with the AIG-93G diet (Fig. 8D).

In addition to NAE levels, 2-monoacylglycerols (2-MAGs) were measured in the same jejunum samples from mice on the AIG-93G diets with different fatty acid compositions. All measured 2-MAGs were present in jejunum at significantly higher levels than the corresponding NAEs (Fig. 9). Feeding induced dramatic elevations in jejunum levels of 2-OG and 2-LG in mice fed with both soybean and lard oil diets (Fig. 9, A and B). The soybean oil diet produced a greater feeding-induced elevation in 2-LG, whereas the lard oil diet produced a greater 2-OG elevation. 2-Palmitoylglycerol and 2-arachidonylglycerol (2-AG) levels increased only in animals fed with the lard oil diet (Fig. 9, C and D).

**DISCUSSION**

A number of fatty acid derivatives have been described as endogenous ligands capable of activating the CB1 and CB2 cannabinoid receptors (26), the two most studied of which, AEA and 2-AG, have been named endocannabinoids. Further
work has revealed the existence of an extended family of endocannabinoid-like endogenous lipids with a wide diversity of biological function (2). Many of these lipids do not actually activate cannabinoid receptors, but they share with true endocannabinoids the same biosynthesis and catabolic pathways (23). The discovery of an increasing number of endocannabinoid-like lipids and their pleiotropic effects on multiple molecular targets has made it challenging to understand the physiological roles of these endogenous compounds.

Early efforts to deorphanize the GPR119 receptor first resulted in the identification of an endocannabinoid-like lipid, OEA, as a likely candidate, based on its potent stimulation of the receptor in vitro and presence in the intestine (16, 25, 27). Subsequent reports provided evidence that compounds structurally related to OEA might also be important regulators of GPR119 receptor activity (8, 18, 19), but no one has reported a complete and thorough evaluation of whether other endogenous endocannabinoid-like lipids can serve as GPR119 ligands. We therefore undertook such a study focusing on compound properties critical for endogenous receptor activation: 1) ability of the ligand to activate GPR119 receptor; 2) translation of the receptor activation into GLP-1 secretion in enteroendocrine cells; 3) the presence of the ligand in the intestinal tissue at levels capable of activating the receptor; and 4) changes in the intestinal ligand concentrations in response to changes in the nutrient load. Our data clearly demonstrate that GPR119 is a fairly promiscuous receptor that can be activated by multiple lipids.

The three most potent lipid GPR119 agonists identified were NAEs (OEA, LEA, and POEA). Interestingly, these three ligands are all amides of mono- or diunsaturated fatty acids containing a 9Z (-cis) carbon-carbon double bond. Saturation of this bond, or introduction of more than two unsaturated bonds in a lipid, resulted in a significant loss of activity at the GPR119 receptor. From our studies, it is also clear that ethanolamine is a preferred polar group for activation of the receptor. Replacement of ethanolamine with any other polar group radically decreased either the GPR119 potency or efficacy of the studied lipids.

We also describe here the GPR119 receptor antagonist arvanil, a synthetic analog of AEA. Arvanil blocked GPR119 agonist-induced increases in cAMP and GLP-1 secretion. To our knowledge, arvanil is the first reported GPR119 antagonist, although it should be noted that it is several orders of magnitude less potent at GPR119 than it is at TRPV1, and one order of magnitude less potent at CB1. However, even with these limitations, arvanil could still be a useful tool in the characterization of GPR119 receptor pharmacology and biology.

Recently, the TRPV1 channel has been implicated in the regulation of GLP-1 secretion, and the TRPV1 agonist capsaicin has been reported to trigger GLP-1 secretion in STC-1 cells (34). In GLUTag cells, we did not see a significant modulation of GLP-1 secretion with arvanil under basal conditions, whereas the selective TRPV1 antagonist SB-366791 inhibited basal GLP-1 secretion. These data suggest that GLUTag cells display high basal TRPV1 activity, and further channel activation with TRPV1 agonists does not lead to significant elevation in GLP-1 secretion.

Activation of GPR119 with OEA and LEA has been reported previously (8, 25); however, we are the first to show that
POEA, produced endogenously from the monosaturated fatty acid palmitoleic acid, is also a potent GPR119 agonist. Palmitoleic acid, abundant in adipose tissue and liver (5), is postulated to play a regulatory role as a major signaling lipid produced from adipose tissue given that elevated levels result in improved hepatic lipid metabolism and enhanced insulin sensitivity. In fact, administration of palmitoleic acid to diabetic animals alleviates hyperglycemia and hyperlipidemia (5). Further studies are required to determine whether any of these effects are mediated through activation of the GPR119 receptor by POEA derived from palmitoleic acid.

To be an endogenous ligand for a receptor, a compound must be generated by a relevant tissue in response to an appropriate stimulus such that sufficient concentrations of the ligand are made available at the receptor to activate it and evoke the receptor-mediated physiological response. Because the GPR119 receptor is proposed to play an important role in nutrient-stimulated incretin hormone secretion from intestinal L cells (7, 15, 25), we studied changes in the abundance of NAEs and 2-MAGs in mouse intestine in response to nutrient intake. Our data suggest that elevations in the intestinal endocannabinoid-like lipids in response to food intake are mostly driven by consumption of fat present in the diet, consistent with previous observations (11, 30).

This study demonstrates that intestinal levels of three lipid GPR119 receptor agonists (OEA, LEA, and 2-OG) increase after food consumption. Increases in OEA and LEA concentrations in the proximal intestine after food consumption as well as high relative intestinal LEA levels at any nutritional state are consistent with earlier reports (1, 16, 27). Our data underline the great excess of fed LEA levels over that of all other intestinal NAES measured. In jejunum, fed LEA levels were more than 12-fold higher than fed levels of OEA or PEA. Jejunum LEA levels were also higher than OEA levels, even in animals receiving oleic acid as a principal dietary fat component. Moreover, LEA is the only NAE that is elevated in the fed state in the distal part of the intestine, where levels of the GPR119 receptor and GLP-1 are highest. Strikingly, LEA is a predominant NAE species only in intestine, whereas, in other tissues, such as brain and liver, LEA levels are typically lower than OEA levels (1). NAES are mostly synthesized from their precursors, N-acylphosphatidylethanolamines (NAPEs), through enzymatic hydrolysis. Linoleoyl containing NAPEs were the most abundant NAPEs detected in intestine (27), which could probably explain the relatively high intestinal LEA levels.

Of the three lipid GPR119 receptor agonists whose intestinal levels increased after food consumption, it is least likely that endogenous OEA can activate the receptor under normal physiological conditions. Assuming that one gram of tissue would have one milliliter volume, in the fed state the aggregate concentration of OEA in the intestine (ca. 70 nM) would be inadequate to activate the receptor based on its in vitro agonist sensitivity, obesity, and inflammation.

DISCLOSURES
All authors are employed by Eli Lilly and Company.

AUTHOR CONTRIBUTIONS

REFERENCES


