

# GPR119 Is Essential for Oleoylethanolamide-Induced Glucagon-Like Peptide-1 Secretion From the Intestinal Enteroendocrine L-Cell

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**OBJECTIVE**—Intestinal L-cells secrete the incretin glucagon-like peptide-1 (GLP-1) in response to ingestion of nutrients, especially long-chain fatty acids. The G $\alpha$ s-coupled receptor GPR119 binds the long-chain fatty acid derivate oleoylethanolamide (OEA), and GPR119 agonists enhance GLP-1 secretion. We therefore hypothesized that OEA stimulates GLP-1 release through a GPR119-dependent mechanism.

**RESEARCH DESIGN AND METHODS**—Murine (m) GLUTag, human (h) NCI-H716, and primary fetal rat intestinal L-cell models were used for RT-PCR and for cAMP and GLP-1 radioimmunoassay. Anesthetized rats received intravenous or intraleal OEA, and plasma bioactive GLP-1, insulin, and glucose levels were determined by enzyme-linked immunosorbent assay or glucose analyzer.

**RESULTS**—GPR119 messenger RNA was detected in all L-cell models. OEA treatment (10  $\mu$ mol/l) of mGLUTag cells increased cAMP levels ( $P < 0.05$ ) and GLP-1 secretion ( $P < 0.001$ ) in all models, with desensitization of the secretory response at higher concentrations. GLP-1 secretion was further enhanced by prevention of OEA degradation using the fatty acid amide hydrolase inhibitor, URB597 ( $P < 0.05$ – $0.001$  vs. OEA alone), and was abolished by H89-induced inhibition of protein kinase A. OEA-induced cAMP levels and GLP-1 secretion were significantly reduced in mGLUTag cells transfected with GPR119-specific small interfering RNA ( $P < 0.05$ ). Application of OEA (10  $\mu$ mol/l) directly into the rat ileum, but not intravenously, increased plasma bioactive GLP-1 levels in euglycemic animals by 1.5-fold ( $P < 0.05$ ) and insulin levels by 3.9-fold ( $P < 0.01$ ) but only in the presence of hyperglycemia.

**CONCLUSIONS**—The results of these studies demonstrate, for the first time, that OEA increases GLP-1 secretion from intestinal L-cells through activation of the novel GPR119 fatty acid derivate receptor *in vitro* and *in vivo*. *Diabetes* 58:1058–1066, 2009

**G**lucagon-like peptide-1 (GLP-1) is an intestinal hormone with potent insulinotropic effects that are essential to the maintenance of normal glucose homeostasis (1). In addition to glucose-dependent stimulation of insulin secretion, GLP-1 shows other favorable effects, increasing  $\beta$ -cell proliferation in

rodents, as well as enhancing  $\beta$ -cell survival in both rodent and in human islets (2,3). Additionally, GLP-1 has been shown to protect cardiomyocytes from ischemia, and GLP-1 infusion improves cardiac function in patients with heart failure (4,5). Finally, the central nervous system effects of GLP-1 include inhibition of gastric emptying, reduction of appetite, and promotion of satiety in humans (6,7) and rodents (8,9). As a result of its potent antidiabetic and anorexic effects, GLP-1 analogs and GLP-1 degradation inhibitors have been successfully introduced to the clinic for pharmacologic treatment of patients with type 2 diabetes (10).

While the biological effects of GLP-1 have been well established, the mechanisms underlying GLP-1 secretion are less well understood. GLP-1 is secreted from intestinal endocrine L-cells, localized predominantly in the distal ileum and colon (11). Rapid GLP-1 release after food intake (12,13) may be regulated by afferent innervation by the vagus nerve (14,15) as well as, in rodents, by proximal gut hormones, such as glucose-dependent insulinotropic peptide (GIP) from the duodenal K-cells (16). However, L-cells also release GLP-1 in response to direct stimulation by nutrients (16), such as carbohydrates and, most notably, fat (17,18). Monounsaturated long-chain fatty acids, such as oleic acid, are strong stimulators of GLP-1 secretion from the L-cells, both *in vitro* and *in vivo*, through a signaling pathway that requires protein kinase C (PKC)  $\zeta$  (17,18). Additional studies have indicated roles for the orphan G-protein-coupled receptors, GPR40 and GPR120, in the response of the L-cell to saturated fat and  $\alpha$ -linolenic acid, respectively (19,20). Very recently, the fatty acid derivate receptor, GPR119, was also found to be expressed in a highly tissue-specific fashion by the intestinal L-cell and the pancreatic  $\beta$ -cell (21,22). Furthermore, a GPR119-specific pharmacological agonist was demonstrated to increase the plasma levels of both GLP-1 and insulin in mice. However, the relevance of physiological ligands of GPR119 to GLP-1 secretion by the L-cell currently remains unknown.

Oleoylethanolamide (OEA) and lysophosphatidylcholine (LPC) are endogenously occurring fatty acid derivatives that are specific ligands of GPR119 (23,24). While LPC is often associated with pathophysiological processes such as atherosclerosis (25), OEA is found in a variety of tissues, including the intestinal epithelium, under physiological conditions (26). OEA is synthesized *in vivo* from membrane phospholipids through an *N*-acylphosphatidylethanolamine (NAPE)-phospholipase D (PLD)-dependent pathway (27); OEA can also be degraded into oleic acid and ethanolamide by the naturally occurring enzyme fatty acid amide hydrolase (FAAH), which is also expressed by the intestinal epithelium (28,29). Intestinal OEA levels are known to decrease during fasting and

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TABLE 1  
PCR primers

Gene (GenBank accession no.)	Primers (5'–3')	Position	Amplicon (bp)
m/rGPR119 (NM_181751)	TGATGGTGTGGCCTTTGCTTCAC TGGTAAAGGCAGCATTTGTGGCAG	328–351 993–970	666
hGPR119 (NM_178471)	TCTCGGCCACACAGAAGA GCTGCGGAGGAAGTGACAAA	208–226 269–250	62
mGPR40 (NM_194057)	TCTGATCTCCTACTGGCCATCACT AGGTCCGGGTTTATGAAACTAGCC	151–175 734–758	608
hGPR40 (NM_005303)	TTCAGCCTCTCTCTCTGCTCTTT TTCTTGCCGCACACACTGTCTTCA	550–573 877–854	328
mGPR120 (NM_181748)	TTTCTTCTCGGATGTCAAGGGCGA TCCGCGATGCTTTCGTGATCTGTA	126–149 763–740	638
hGPR120 (NM_181745)	TCGATTTGCACACTGATTTGGCCC AAATGTGAAGCCACCACCCAGAA	630–653 1,037–1,014	408

increase upon refeeding, and OEA administration to rats reduces food intake, suggesting a role for OEA in the regulation of satiety (26,30–32). As OEA was first identified as a ligand for the intranuclear peroxisome proliferator-activated receptor (PPAR)  $\alpha$ , it has been generally assumed that the appetite reduction is dependent on PPAR $\alpha$  activation (33). However, as GLP-1 is known to induce satiety, we hypothesized that OEA may also stimulate GLP-1 secretion from the intestinal L-cells in a GPR119-dependent fashion.

## RESEARCH DESIGN AND METHODS

**Cell models.** The murine (m) GLUTag L-cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 25 mmol/l glucose and supplemented with 10% FBS; the medium was changed every 2–3 days and cells were passaged by trypsinization and reseeding at a 1:3 dilution (17,34,35). The human (h) NCI-H716 L-cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were grown in suspension in RPMI-1640 supplemented with 10% FBS (35,36). Fetal rat intestinal cultures (FRICs) were prepared by enzymatic dispersal of term fetal intestines from 19 to 20 days' pregnant Wistar rats, and cells were maintained overnight in DMEM containing 25 mmol/l glucose, 10% FBS, and penicillin-streptomycin, as previously reported (15,17,35,37). All three models of the intestinal L-cell have been validated with respect to the regulation of GLP-1 secretion, such that GLP-1 is secreted in response to known secretagogues, including muscarinic/cholinergic agonists, leptin and long-chain fatty acids in all models, as well as glucose-dependent insulinotropic peptide (GIP) in the rodent cells (17,34–38).

**In vitro experiments.** mGLUTag cells were plated in poly-D-lysine-coated 24-well culture plates and grown to 80% confluence. For experiments with hNCI-H716 cells, adhesion of the cells was initiated by plating on Matrigel matrix (Becton Dickinson, Bedford, MA) in DMEM medium, supplemented with 10% FBS, 2 days before the experiment, as described (35,38). FRIC cells were investigated the day after cell dispersal and plating. Adenosine 3',5'-cyclic monophosphate (cAMP) responses to OEA were determined by washing mGLUTag cells with Hanks' balanced salt solution followed by treatment for 30 min with FBS-free medium containing 1% DMSO alone (negative control), 10  $\mu$ mol/l forskolin (positive control), or 10–15  $\mu$ mol/l OEA, as previously described (39). To prevent cAMP degradation, all media contained 10  $\mu$ mol/l 3-isobutyl-1-methylxanthine (Sigma-Aldrich, Oakville, ON, Canada). Cells were then extracted in ethanol for radioimmunoassay (RIA) of cAMP content. Secretion experiments were performed as described (17,34–36). In brief, mGLUTag, hNCI-H716, and FRIC cells were washed and then incubated for 2 h with FBS-free DMEM containing 1% DMSO alone (negative control), 10  $\mu$ mol/l forskolin (Sigma-Aldrich), or 1  $\mu$ mol/l GIP (positive controls) (Bachem, Torrance, CA) or with different concentrations of OEA, palmitoylethanolamide (PEA), or LPC (all from Sigma-Aldrich). Some cells were preincubated for 30 min with 10  $\mu$ mol/l or 30  $\mu$ mol/l H89 (Sigma-Aldrich) to inhibit protein kinase A (PKA) or with 1  $\mu$ mol/l URB597 (Calbiochem, Mississauga, ON, Canada), an FAAH inhibitor (29). DMSO was used as a solvent to prepare stock solutions of fatty acid derivatives and inhibitors. For secretion experiments, medium and cells were collected separately and peptides were extracted by reversed-phase adsorption using C<sub>18</sub> silica cartridges (Sep-Pak; Waters Scientific, Mississauga, ON, Canada), as previously

described (17,34–36), for RIA of GLP-1 content. GLP-1 secretion was calculated as total GLP-1 content of medium normalized for the total amount of GLP-1 in the medium plus cells. Average basal secretion of mGLUTa, hNCI-H716, and FRIC cells was  $3.4 \pm 0.3\%$  ( $n = 31$ ),  $2.7 \pm 0.4\%$  ( $n = 28$ ), and  $2.86 \pm 0.6\%$  ( $n = 4$ ), respectively, of total GLP-1. No changes in total GLP-1 content were found under any of the experimental conditions (data not shown).

**Small interfering RNA transfection.** mGLUTag cells were plated in a 24-well plate, as described above. Scrambled small interfering RNA (siRNA) (control) and two siRNAs targeting murine GPR119 coding sequences were purchased from Ambion (Austin, TX). Transfection was performed by 5-h incubation in Opti-Mem medium using 20 pmol/l siRNA and 1  $\mu$ l Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) as instructed by the manufacturer. Cells were allowed to recover for 2 days before cAMP and/or secretion experiments. Transfection efficiency was quantified by real-time RT-PCR. In brief, total RNA was extracted from mGLUTag cells using an RNeasy kit as instructed by the manufacturer (Qiagen, Mississauga, ON, Canada) and subjected to reverse transcription using SuperScript II and random hexamers (Invitrogen), followed by real-time PCR using TaqMan gene expression assays (Applied Biosystems, Foster City, CA) for GPR119 (Mm00731497\_s1) and 18S (Hs99999901\_s1; endogenous control). Relative quantification of GPR119 mRNA expression was calculated using the  $\Delta\Delta$  cycle threshold method (40).

**In vitro assays.** Viability of the mGLUTag cells after treatment was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay, as reported (17). Cells were plated in poly-D-lysine-coated 96-well plates and treated for 2 h with medium containing 1% DMSO alone (control), 5 mmol/l H<sub>2</sub>O<sub>2</sub> (positive control), or fatty acid derivatives or inhibitors at concentrations used for the secretion experiment, after which the MTT reaction was carried out. The resulting absorbance was measured at 570 nm; higher absorbance correlates with cell viability and lower absorbance with cell death. cAMP was measured by RIA of ethanol extracts as described (39) (Biomedical Technologies, Stoughton, MA). RIA for COOH-terminal GLP-1 immunoreactivity was conducted using an established lab assay (17,34–36).

As there is currently no good GPR119 antiserum commercially available, GPR119 expression was determined by RT-PCR. Human jejunum and colon total RNA was purchased from Ambion, and human placental RNA was a kind gift from Dr. J.R. Challis (University of Toronto, Toronto, ON, Canada). Total RNA from mGLUTag, hNCI-H716, and FRIC cells, as well as from rodent tissues, was extracted as described above. The primer pairs used for amplification of human GPR119 were described by Soga et al. (23) and for mouse GPR40 and GPR120 by Iakoubov et al. (17); all other primer pairs were designed using PrimerQuest (IDT, Coralville, IA) (sequences are shown in Table 1). PCR were carried out at 57°C for 35 cycles, and PCR without RNA template was used as the negative control. Products were analyzed by agarose gel electrophoresis and visualized with ethidium bromide.

**In vivo experiments.** In vivo animal protocols were approved by the University of Toronto Animal Care Committee. Male Wistar rats (200–300 g) were obtained from Charles River Laboratories (St. Constant, QC, Canada) and maintained on a standard laboratory diet with free access to water under a 12-h light-dark schedule. Following an overnight fast, rats were anesthetized with isoflurane (Baxter, Mississauga, ON, Canada). One hour before blood sampling, some rats were intraperitoneally injected with 3 mg/kg URB597 to inhibit FAAH (29,41). URB597 alone did not affect the levels of glucose,

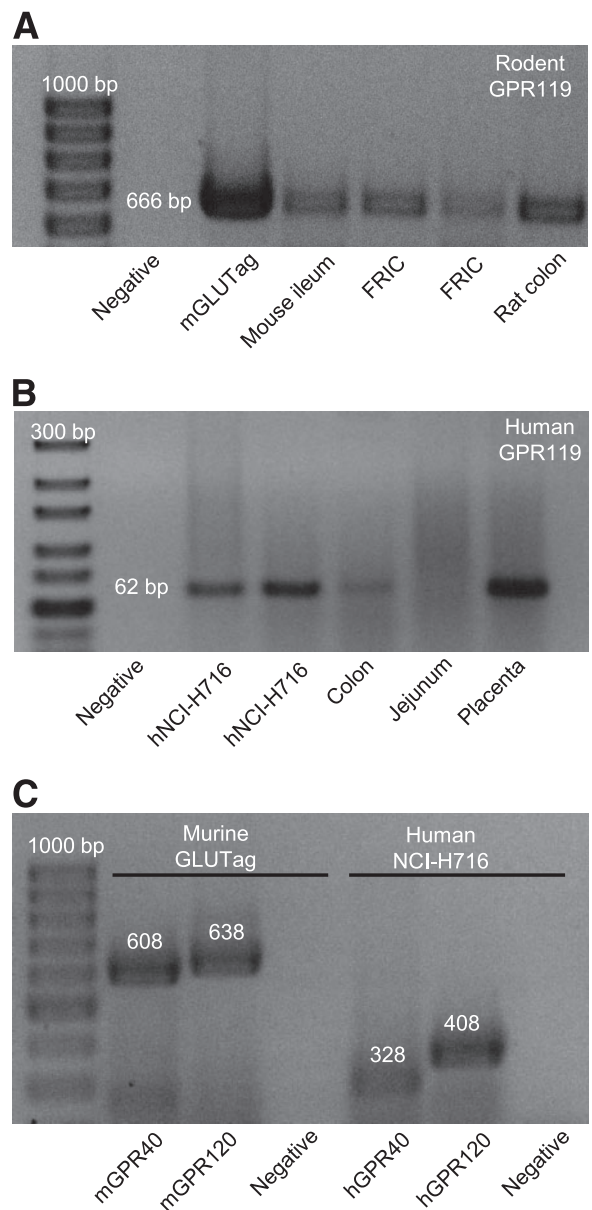
insulin, or bioactive GLP-1 in these studies; therefore, data from rats with and without URB597 injection were combined. The carotid artery was cannulated for blood sampling and the jugular vein for injections. To prevent bioactive GLP-1 degradation by dipeptidylpeptidase (DPP) IV, rats received 5 mg/kg Sitagliptin (Januvia; MSD Sharp & Dohme, Haar, Germany), a selective inhibitor of DPP-IV (10,42), intravenously 30 min before blood collection. Some rats also received a femoral vein cannula for continuous infusion of 37.5% glucose to maintain glycemia at 13 mmol/l for at least 30 min. The glucose infusion rate was adjusted based on frequent (every 5–10 min) blood glucose measurements, as described in Goh et al. (43). Rats undergoing a hyperglycemic clamp were not pretreated with Sitagliptin in order to reduce the number of variables that might affect glycemia. The abdominal cavity of all rats was then opened and a 10-cm section of the distal ileum was cleansed by perfusion with saline and tied off to create a lumenally distinct compartment that retained vascular perfusion. Subsequent to collection of the basal blood sample at  $t = 0$  min, either the luminal compartment was filled with 2 ml of 10  $\mu$ mol/l OEA or vehicle (0.9% saline/10% Tween 80; Sigma-Aldrich), or 5 mg/kg OEA or vehicle (0.9% saline/10% Tween 80) was administered intravenously, and additional blood samples were collected at 5, 15, 30, and 60 min. All samples (1 ml each) were collected into 10% (vol/vol) Trasylol (5,000 Kalikrein inactivating units/ml; Bayer, Toronto, ON, Canada), EDTA (12 mg/ml), and diprotin A (a DPP-IV inhibitor, 68 mg/ml; Sigma-Aldrich), and plasma was stored at  $-80^{\circ}\text{C}$  until analysis. Plasma glucose levels were analyzed on a Beckman Analyzer II (Beckman, Fullerton, CA), and plasma insulin levels were measured by enzyme-linked immunosorbent assay (Crystal Chem, Downers Grove, IL) in normoglycemic animals and by RIA (Millipore, Billerica, MA) in hyperglycemic animals due to the wider insulin detection range. Plasma levels of bioactive GLP-1 were determined by electrochemiluminescence-based detection assay (Meso Scale Discovery, Gaithersburg, MD).

**Statistical analyses.** All results are expressed as means  $\pm$  SE. Area under the curve (AUC) was determined using the trapezoidal rule, and the data are expressed per min. Statistical analysis was performed using SAS software (SAS Institute, Cary, NC). One- and two-way ANOVA was followed by Student's  $t$  test or  $n-1$  custom hypotheses post hoc tests, as appropriate. To reduce interassay variations, some data were normalized to basal levels. Significance was assumed at  $P < 0.05$ .

## RESULTS

**Expression of GPR40, GPR119, and GPR120.** RT-PCR for rodent GPR119 mRNA transcripts was performed on total RNA extracted from mGLUTag and FRIC cells, as well as from mouse ileum and rat colon tissue. As shown in Fig. 1A, GPR119 mRNA was detected in both rodent L-cell models, as well as in the intestinal samples. Additionally, GPR119 mRNA was detected in the hNCI-H716 cells and in human colon and placental tissue (Fig. 1B). In contrast to previous reports (22), we did not detect GPR119 mRNA in human jejunum, possibly due to a low number of L-cells in this tissue (11). Consistent with our previous findings (17), mRNA transcripts for both GPR40 and GPR120 were detected in the mGLUTag cells, and both receptor mRNAs were also found to be expressed in the hNCI-H716 cells (Fig. 1C).

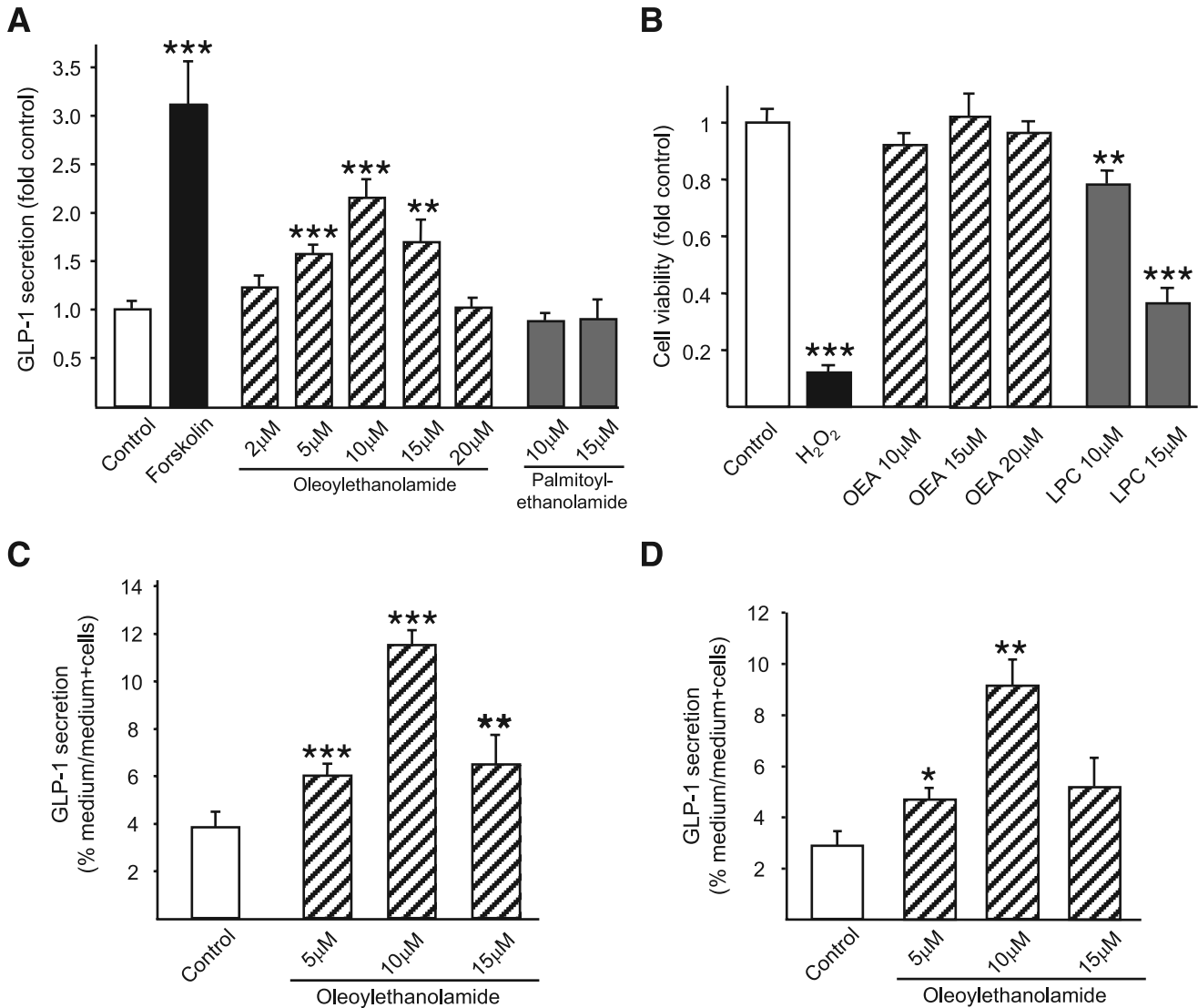
**OEA induces GLP-1 secretion in vitro.** Possible effects of GPR119 receptor activation on GLP-1 secretion were first investigated in mGLUTag cells. Release of GLP-1 was increased to  $3.1 \pm 0.4$ -fold of basal values by treatment with forskolin, a direct activator of adenylyl cyclase and strong L-cell secretagogue (34). OEA (5–20  $\mu$ mol/l), a known ligand of GPR119 (24), significantly increased GLP-1 secretion to a maximum of  $2.1 \pm 0.2$ -fold of basal levels at 10  $\mu$ mol/l ( $P < 0.001$ ) (Fig. 2A). The same concentrations of PEA, a saturated fatty acid ethanolamide (16:0) that is a very weak agonist of GPR119 (23,24), did not increase GLP-1 secretion from mGLUTag cells. Importantly, OEA treatment did not affect the viability of the GLUTag cells (Fig. 2B). In contrast, although LPC treatment of mGLUTag cells at concentrations reported to activate GPR119 (10–15  $\mu$ mol/l) also enhanced GLP-1 release (to a maximum of  $10.4 \pm 0.5$ -fold of control values;



**FIG. 1.** Expression of GPR119 mRNA in L-cell models. **A:** Total RNA from mGLUTag cells, FRICs, and murine intestinal tissues was analyzed for expression of GPR119 mRNA by RT-PCR. **B:** Total RNA from hNCI-H716 cells, human intestinal tissues, and human placenta was analyzed for expression of GPR119 mRNA by RT-PCR. **C:** Total RNA from mGLUTag cells and hNCI-H716 cells was analyzed for expression of GPR40 and GPR120 mRNA by RT-PCR. All products were separated on agarose gels and visualized with ethidium bromide, with the molecular-size ladder on the left. Negative controls did not include RNA template. The anticipated band sizes of products are indicated in base pairs (bp).

data not shown), LPC was found to markedly reduce cell viability by up to  $60.0 \pm 6.8\%$  compared with control cells ( $P < 0.05$  and  $P < 0.001$ ) (Fig. 2B); LPC was, therefore, not used in further experiments. The effects of OEA on GLP-1 secretion were also confirmed in hNCI-H716 and FRIC cells (Fig. 2C and D), wherein OEA treatment significantly increased GLP-1 secretion to  $2.6 \pm 0.2$ - and  $5.8 \pm 2.5$ -fold of basal levels at 10  $\mu$ mol/l ( $P < 0.001$  and  $P < 0.01$ ), respectively. Interestingly, higher concentrations of OEA were associated with diminished GLP-1 release in all cell models, suggestive of desensitization.

To verify the activity of GPR119 in the L-cell, mGLUTag and hNCI-H716 cells were treated with a specific GPR119



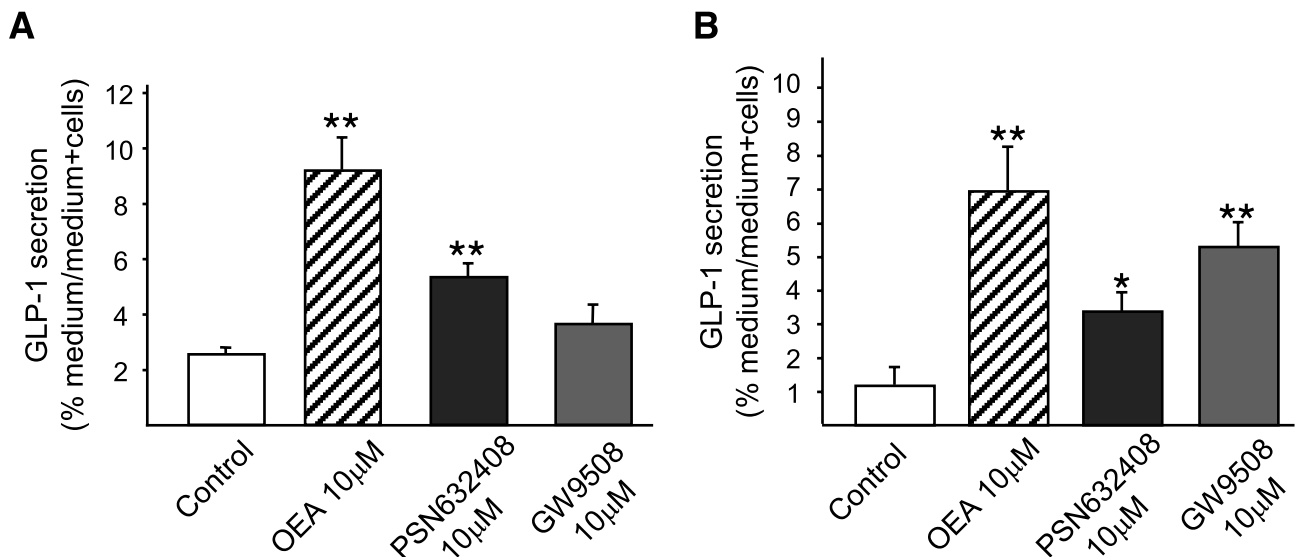
**FIG. 2.** Effects of OEA on GLP-1 secretion. mGLUTag cells ( $n = 9-12$ ) (A), hNCI-H716 cells ( $n = 8$ ) (C), and FRIC cells ( $n = 4$ ) (D) were incubated with medium alone (1% DMSO, negative control), forskolin (10  $\mu\text{mol/l}$ , positive control), OEA (2–20  $\mu\text{mol/l}$ ), or PEA (10–15  $\mu\text{mol/l}$ , negative control) for 2 h. GLP-1 content of media and cells was determined by RIA. B: To determine potential effects on cell viability, mGLUTag cells were incubated with medium alone (1% DMSO, negative control), H<sub>2</sub>O<sub>2</sub> (5 mmol/l, positive control), OEA (10–20  $\mu\text{mol/l}$ ), or LPC (10–15  $\mu\text{mol/l}$ ) for 2 h, followed by MTT assay ( $n = 8-16$ ). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  vs. control.

agonist, PSN632408 (10  $\mu\text{mol/l}$ ) (24). PSN632408 increased GLP-1 secretion by both cell lines to  $2.1 \pm 0.2$ - and  $2.9 \pm 0.5$ -fold of basal values ( $P < 0.01$  for mGLUTag and  $P < 0.05$  for hNCI-H716 cells) (Fig. 3), demonstrating that GPR119 is functional and can initiate GLP-1 secretion in these cells. To determine possible function of the other fatty acid receptors expressed in the L-cell lines GPR40 and GPR120 (Fig. 1C), both mGLUTag and hNCI-H716 cells were also treated with the combined GPR40/GPR120 agonist GW9508 (10  $\mu\text{mol/l}$ ) (24). Consistent with previous findings from our group (17), GPR40 and GPR120 were not found to be involved in GLP-1 secretion from mGLUTag cells, as treatment with GW9508 did not increase GLP-1 secretion (Fig. 3). In contrast, GW9508 increased GLP-1 secretion by hNCI-H716 cells to  $4.5 \pm 0.6$ -fold of basal values ( $P < 0.01$ ), providing indication for a role of GPR40 and/or GPR120 in human L-cells.

Prevention of OEA degradation with URB597 significantly increased OEA-induced GLP-1 secretion to  $3.2 \pm 0.4$ - and  $3.3 \pm 0.3$ -fold of control values at 10 and 15  $\mu\text{mol/l}$

OEA in mGLUTag cells ( $P < 0.001$  compared with URB597 alone and  $P < 0.01-0.001$  compared with OEA treatment without URB597) (Fig. 4A), as well as in hNCI-H716 cells (to  $3.5 \pm 0.2$ - and  $5.5 \pm 0.7$ -fold of control values at 10 and 15  $\mu\text{mol/l}$  OEA ( $P < 0.001$  compared with URB597 alone and  $P < 0.05-0.001$  compared with OEA treatment without URB597) (Fig. 4B). These findings indicate that degradation by FAAH limits the effects of OEA on GLP-1 secretion in both of the intestinal L-cell lines utilized.

**OEA signals through a PKA- and GPR119-dependent mechanism.** As ligand binding to GPR119 leads to activation of adenylyl cyclase, increased production of cAMP, and enhanced PKA activity (24), mGLUTag cells were first examined for cAMP responses to OEA (10–15  $\mu\text{mol/l}$ ) and GIP, which is known to signal through cAMP- and PKA-dependent pathways (positive control) (44). OEA treatment alone caused a small but significant increase in intracellular cAMP to  $1.11 \pm 0.03$ - and  $1.12 \pm 0.04$ -fold of control values at 10 and 15  $\mu\text{mol/l}$ , respectively ( $P < 0.05$ ) (Fig. 5A). This effect was enhanced when OEA degradation



**FIG. 3.** Effects of GPR119 and GPR40/120 agonists on GLP-1 secretion. mGLUTag ( $n = 4$ ) (A) and hNCI-H716 ( $n = 4$ ) (B) cells were incubated with medium alone (1% DMSO, negative control), OEA (10  $\mu\text{mol/l}$ ), the GPR119 agonist PSN632408 (10  $\mu\text{mol/l}$ ), or the combined GPR40/GPR120 agonist GW9508 (10  $\mu\text{mol/l}$ ) for 2 h. GLP-1 content of media and cells was determined by RIA. \* $P < 0.05$ ; \*\* $P < 0.01$  vs. control.

was prevented with URB597, such that cAMP levels increased by an additional  $1.3 \pm 0.04$ – and  $1.3 \pm 0.03$ –fold, respectively ( $P < 0.001$  compared with URB597 alone and  $P < 0.05$ – $0.01$  compared with OEA treatment without URB597). Furthermore, pretreatment of the mGLUTag cells with the PKA inhibitor H89 (10  $\mu\text{mol/l}$ ) completely abolished OEA (10–15  $\mu\text{mol/l}$ )-induced GLP-1 secretion (Fig. 5B). Similar results were found in hNCI-H716 cells, although an increased concentration of H89 (30  $\mu\text{mol/l}$ ) was required to abrogate OEA-induced GLP-1 release (Fig. 5C). H89 treatment did not affect GPR40/120-induced GLP-1 secretion (Fig. 5C, inset), demonstrating the specificity of this inhibitor for PKA-mediated signaling.

To determine whether the effects of OEA on cAMP production and GLP-1 secretion are dependent upon GPR119, mGLUTag cells were transfected with specific GPR119 siRNA or scrambled siRNA (control), resulting in 23% knockdown of GPR119 mRNA, as determined by real-time RT-PCR (Fig. 6A, inset). Despite the relatively low level of GPR119 knockdown, OEA (10  $\mu\text{mol/l}$ ) failed to enhance cAMP levels in cells treated with GPR119 siRNA, whereas the cAMP response to OEA treatment was preserved in the control cells ( $P < 0.05$ ) (Fig. 6A). Furthermore, GPR119 knockdown led to a 45% reduction in the GLP-1 secretory response to OEA ( $P < 0.05$ ) (Fig. 5B). These data therefore provide support for a role of GPR119 and the PKA signaling pathway in OEA-induced GLP-1 secretion.

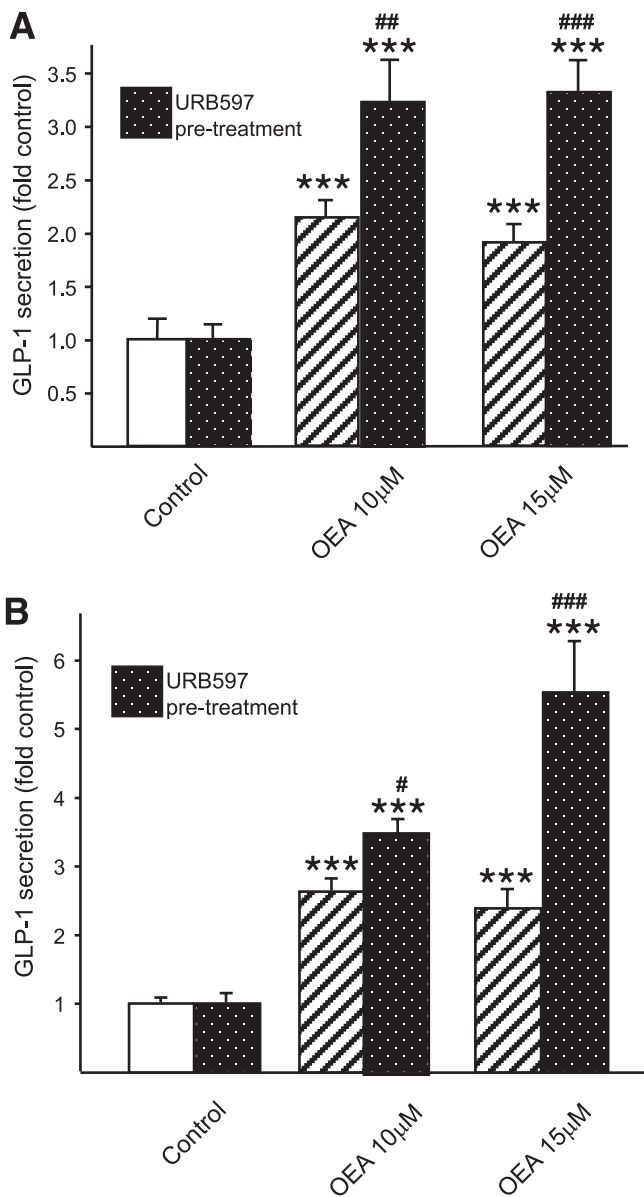
**OEA enhances GLP-1 secretion in vivo.** To establish the effects of OEA on the L-cell in vivo, rats were treated with OEA either intraluminally or intravenously. Intraluminal application of OEA (10  $\mu\text{mol/l}$ ; e.g., 20 nmol/rat) to euglycemic rats significantly increased plasma bioactive GLP-1 concentrations to  $1.5 \pm 0.2$ –fold of basal values ( $P < 0.05$ ) within 5 min of administration, and this stimulation was maintained throughout the entire 60-min time course of the experiment (Fig. 7A). Thus, the AUC for the bioactive GLP-1 response was significantly increased to  $1.6 \pm 0.1$ –fold of vehicle-infused rats ( $P < 0.001$ ) (Fig. 7B). In contrast, intravenous administration of OEA at a 200-fold-higher dose (e.g., 4  $\mu\text{mol}/250$  g rat) than that

used intraluminally demonstrated no effect on bioactive GLP-1 concentrations compared with rats treated with vehicle alone. Throughout the 60-min experiment, the plasma levels of glucose (Fig. 7C) and insulin (Fig. 7D) remained stable at basal levels and did not differ between treatment and control groups.

As GLP-1 is known to lose its insulinotropic effects under normoglycemic conditions, changes in insulin levels upon OEA treatment were also measured under hyperglycemic conditions. Glycemia was maintained at 13 mmol/l, the upper physiological level in rats, for at least 30 min before the start of OEA application and throughout the remainder of the procedure. The basal concentration of insulin before OEA application was  $1.4 \pm 0.4$  pg/ml and did not differ between the groups. Intraluminal application of OEA caused a  $3.9 \pm 0.7$ –fold increase in insulin plasma levels within 5 min of application ( $11.2 \pm 2.1$  vs.  $2.8 \pm 2.0$  pg/ml in the control group;  $P < 0.01$ ) (Fig. 7D, inset). In contrast, intravenous infusion of OEA did not affect insulin levels during the entire treatment period in the hyperglycemic rats.

## DISCUSSION

Previous studies have indicated that the fatty acid derivate receptor GPR119 is present on pancreatic  $\beta$ -cells and intestinal L-cells, and its stimulation by a GPR119 agonist increases insulin and GLP-1 secretion, respectively (21,22,24). However, the role of physiologically occurring ligands of GPR119, such as OEA, and the intracellular mechanisms underlying GPR119-dependent GLP-1 secretion from the intestinal L-cell have remained undefined. The results of the present study demonstrate, for the first time, that OEA stimulates GLP-1 secretion from both mouse and human intestinal L-cell lines, as well as from primary rat L-cells in vitro. Additionally, application of OEA directly into the intestinal lumen in rats induced a significant and persistent increase in bioactive GLP-1 levels over 1 h, supporting the in vitro findings and demonstrating a role for OEA as a GLP-1 secretagogue in vivo.



**FIG. 4.** Effect of inhibition of OEA degradation on OEA-induced GLP-1 secretion. mGLUTag ( $n = 6-18$ ) (A) and hNCI-H716 ( $n = 12$ ) (B) cells were pretreated for 30 min with URB597 (1  $\mu\text{mol/l}$ ) to inhibit FAAH and prevent OEA degradation before incubation with medium alone (1% DMSO, negative control) or OEA (10–15  $\mu\text{mol/l}$ ) for 2 h. GLP-1 content of media and cells was determined by RIA. \*\*\* $P < 0.001$  vs. control; # $P < 0.05$ ; ## $P < 0.01$ ; ### $P < 0.001$  vs. OEA treatment alone.

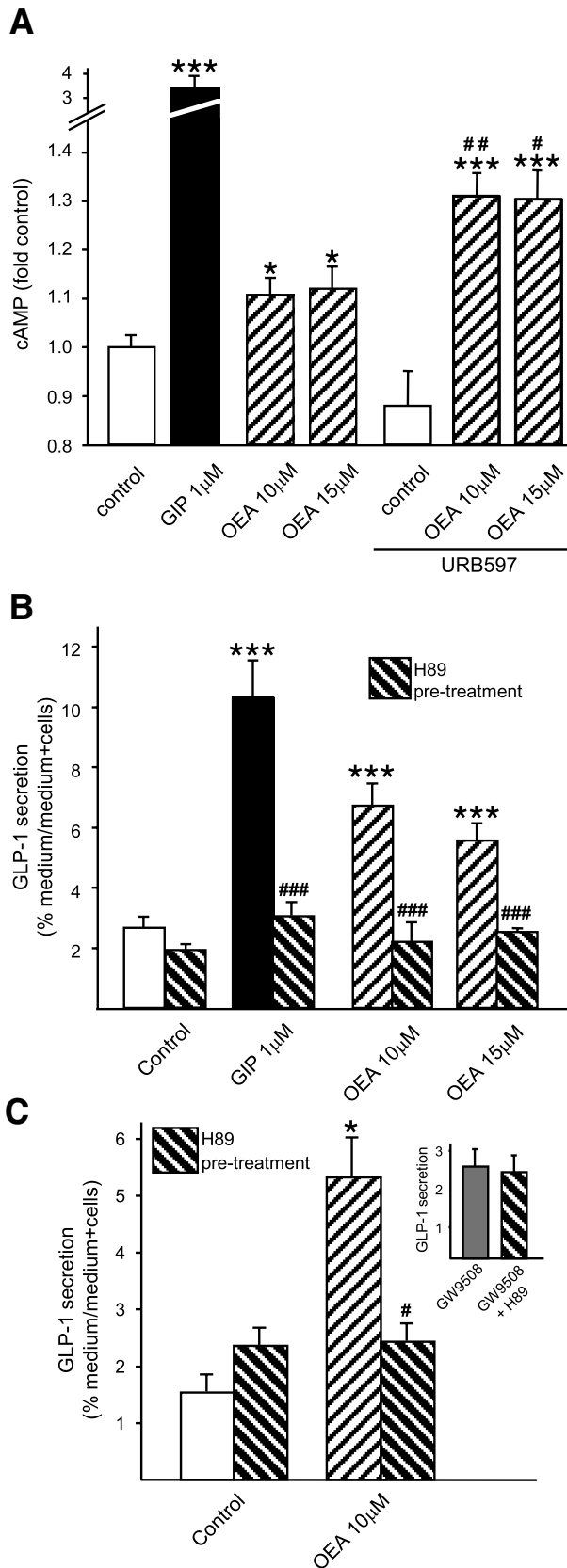
To further establish the role of OEA in GLP-1 secretion, the intracellular signaling mechanisms underlying its effects on the L-cell were investigated. OEA induced a small, but significant, increase in intracellular cAMP concentration in both the mGLUTag and hNCI-H716 cells, comparable with findings in OEA-treated RINm5 and MIN6 pancreatic  $\beta$ -cell lines (45). Furthermore, GLP-1 secretion in OEA-treated cells was strictly dependent on PKA in both the human and mouse L-cells, as indicated by complete abrogation of the response in H89-treated cells. These findings are consistent with studies by our lab and others showing that increased cAMP levels, in response to cAMP analogs as well as to secretagogues such as GIP, stimulate GLP-1 release by the intestinal L-cell (16,34,46). Furthermore, while initial studies on OEA identified the intranuclear receptor PPAR $\alpha$  and transient receptor po-

tential vanilloid type 1 (TRPV1) as targets for OEA (27,33,47), neither of these receptors has been reported to stimulate the cAMP/PKA signaling pathway. In contrast, the deorphanization of GPR119 as a cAMP-linked OEA receptor (24) implicated GPR119 as more likely target for OEA in the intestinal L-cell. Consistent with this hypothesis, treatment of human and mouse intestinal L-cells with a GPR119-specific agonist PSN632408 significantly increased GLP-1 secretion in both cell lines. Furthermore, transfection of mGLUTag cells with GPR119 siRNA significantly diminished both the cAMP and GLP-1 responses to OEA. When taken together, these findings provide support for both the presence of functional GPR119 in the intestinal L-cell and the requirement for this novel G-protein-coupled receptor in OEA-induced GLP-1 secretion.

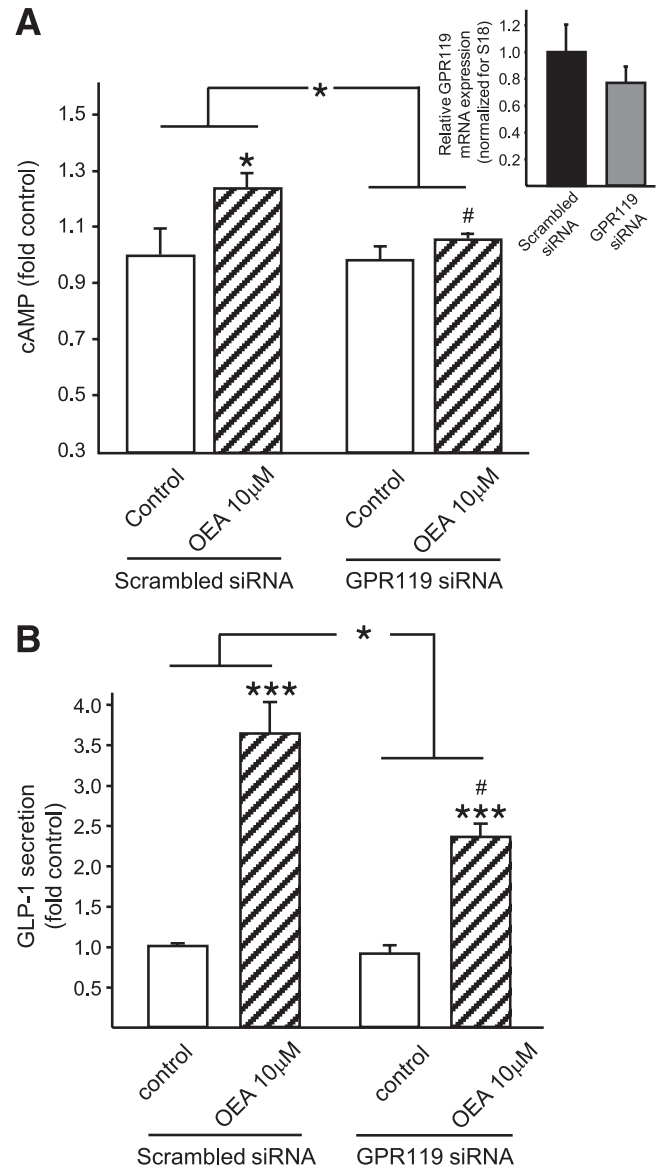
We have previously reported that oleic acid is a strong L-cell secretagogue, increasing GLP-1 secretion both in vivo and in vitro (17,18,48). It is therefore interesting that OEA is rapidly degraded to oleic acid and ethanolamide via FAAH-dependent hydrolysis (29), thereby providing a possible GPR119-independent mechanism underlying OEA-induced GLP-1 secretion. Therefore, to exclude the possible effects of oleic acid on the actions of OEA, L-cells were pretreated with the FAAH inhibitor URB597 to reduce OEA hydrolysis and resultant oleic acid accumulation. The increase in OEA-induced GLP-1 secretion found with URB597 treatment provides support for a direct role of OEA, and not of oleic acid, in OEA-induced GLP-1 secretion.

While intraluminal application of OEA in vivo significantly increased GLP-1 secretion in normal rats, intravenous injection of OEA at a 200-fold-higher dose failed to increase circulating levels of bioactive GLP-1. Additionally, while intraluminal OEA application clearly increased insulin levels in hyperglycemic rats, intravenous injection of OEA did not affect insulin levels in these animals under either euglycemic or hyperglycemic conditions. Taken together, this data suggests that circulating OEA does not cause significant increases in hormone release from either of its known target tissues (the intestinal L-cell and the pancreatic  $\beta$ -cell). There are several possible explanations for these findings. First, the volume of distribution for OEA as a lipophilic substance should be high, therefore increasing the concentration of OEA required for administration into the jugular vein to reach distant target tissues at stimulatory concentrations. However, higher levels of OEA cannot be achieved in the circulation of the rat due to the limited solubility of OEA in solvents. Furthermore, whether OEA is cleared through the liver and/or lungs is unknown and may be significant.

The actions of both OEA and a GPR119 agonist on insulin secretion have previously been reported to be glucose dependent (21,45), as further confirmed in the present study. Previous studies have also demonstrated that ~45% of the glucose-lowering effect of an oral GPR119 agonist given concurrently with oral glucose is mediated through enhancement of GLP-1 release (22), suggesting that the insulinotropic effect of GPR119 activation is mediated both directly, through GPR119 expressed on the  $\beta$ -cell, and indirectly, through enhanced release of GLP-1. Nonetheless, although both the L-cell and the  $\beta$ -cell are responsive to OEA, the glucose sensitivity of the  $\beta$ -cell response, compared with the glucose insensitivity of the L-cell response that we have observed, suggests that oral administration of this fatty acid derivative alone to enhance GLP-1 secretion, without influencing insulin release, will



**FIG. 5.** Effect of PKA inhibition on OEA-induced GLP-1 secretion. mGLUTag ( $n = 6-9$ ) (A and B) and hNCI-H716 ( $n = 4-6$ ) (C) cells were pretreated for 30 min with medium alone (1% DMSO, negative control), H89 (10  $\mu$ M) for mGLUTag and 30  $\mu$ M for hNCI-H716), or URB597 (1  $\mu$ M) to inhibit PKA or FAAH, respectively, followed by incubation with medium alone (1% DMSO, negative control), GIP (1  $\mu$ M), positive control), or OEA (10–15  $\mu$ M) for 2 h. C, inset: hNCI-H716 cells were pretreated for 30 min with media alone (1%

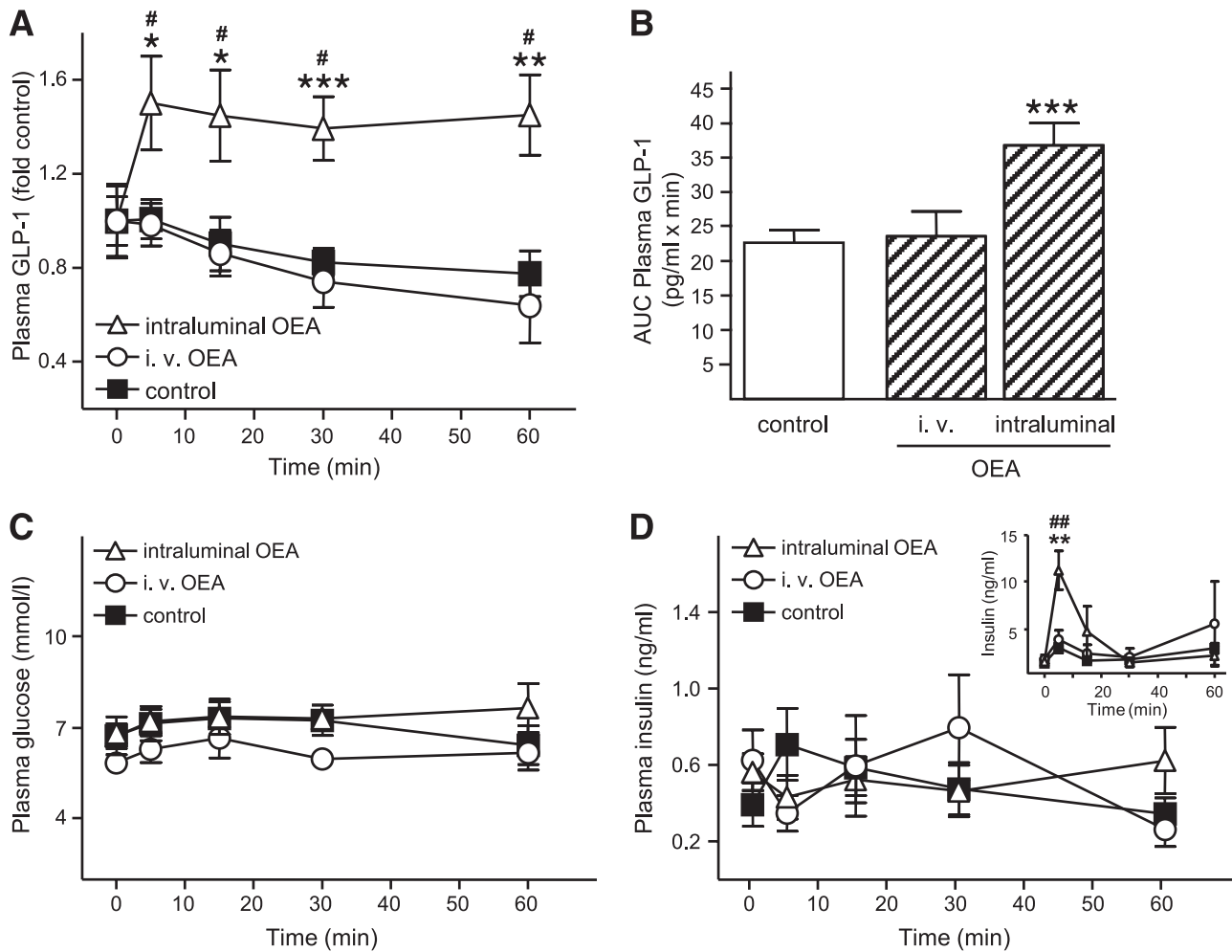


**FIG. 6.** Effect of GPR119 knockdown on OEA-induced GLP-1 secretion. mGLUTag cells were transfected with scrambled siRNA (20 pmol/L, control) or GPR119 siRNA (20 pmol/L) 2 days before the experiment. Cells were then incubated with medium alone (1% DMSO, negative control) or OEA (10–15  $\mu$ M) for 2 h. cAMP content of cells ( $n = 6$ ) (A) and GLP-1 content of media and cells ( $n = 9$ ) (B) were determined by RIA. A, inset: GPR119 mRNA transcript levels were determined by quantitative RT-PCR relative to 18S transcript levels. \* $P < 0.05$ ; \*\*\* $P < 0.001$  vs. control or vs. the  $\Delta$  change in control cells, as indicated by the lines. # $P < 0.05$  vs. OEA treatment with scrambled siRNA.

permit the insulin-independent biological actions of GLP-1. In contrast, administration of OEA in the setting of a glucose-containing meal would facilitate release of both GLP-1 and insulin, thereby also modulating glycemic responses (22,49).

The observation of apparent desensitization in all of the dose-response curves was unexpected, as this has not previously been reported for GPR119. However, G $\alpha$ -coupled receptors are well established to undergo homol-

DMSO) or with H89 (30  $\mu$ M), followed by incubation with the GPR40/120 agonist GW9508 (10  $\mu$ M). cAMP content of cells and GLP-1 content of media and cells were determined by RIA. \* $P < 0.05$ ; \*\*\* $P < 0.001$  vs. appropriate control; # $P < 0.05$ ; ## $P < 0.01$ ; ### $P < 0.001$  vs. paired treatment alone.



**FIG. 7.** In vivo effect of OEA on GLP-1 secretion. Anesthetized rats received intraluminal or intravenous injections of vehicle (saline/10% Tween 80; combined controls), intraluminal OEA (2 ml of 10  $\mu$ mol/l), or intravenous OEA (5 mg/kg), and blood samples were collected over a 1-h period. Plasma concentrations of bioactive GLP-1 (A), glucose (C), and insulin (D) were determined by ELISA and glucose analyzer, as appropriate ( $n = 5-11$ ). B: AUC for the absolute plasma bioactive GLP-1 concentrations was determined using the trapezoidal rule and is expressed per min. D, inset: Rats ( $n = 4-5$ ) were maintained at 13 mmol/l plasma glucose (hyperglycemic clamp) for a minimum of 30 min, and this was maintained throughout the OEA treatment procedure. Plasma insulin levels were determined by RIA. A: To reduce interassay variations due to use of separate kits, bioactive GLP-1 concentrations were calculated as fold increase over basal GLP-1 levels (control:  $29.4 \pm 8.6$  pg/ml; intraluminal OEA:  $18.9 \pm 4.9$  pg/ml; and intravenous OEA:  $32.7 \pm 3.4$  pg/ml;  $P = NS$  between the basal values). \* $P < 0.05$  vs. control; \*\* $P < 0.01$  vs. control; \*\*\* $P < 0.001$  vs. control; # $P < 0.05$  and ## $P < 0.01$  vs. basal values.

ogous desensitization (50); indeed, preliminary studies in which hNCI-716 cells were pretreated with the GPR119 agonist PSN632408 (10  $\mu$ mol/l for 6 h) demonstrated a  $70.9 \pm 4.6\%$  and  $50.7 \pm 11.7\%$  decrease in subsequent OEA- and PSN632408-induced GLP-1 secretion, respectively (data not shown). Collectively, these findings indicate that GPR119 may undergo homologous desensitization, a phenomenon that clearly warrants further investigation.

In summary, the results of this study establish, for the first time, the role of GPR119 in OEA-induced GLP-1 secretion and add GPR119 to the growing list of fatty acid-responsive pathways that function to modulate release of GLP-1, including GPR40, GPR120, and PKC $\zeta$ . When combined with the reported insulinotropic effects of GPR119 agonists, these findings implicate GPR119 as a potential pharmacological target, as well as OEA as a nutraceutical approach to enhance GLP-1 in patients with type 2 diabetes.

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