Postprandial inhibition of gastric ghrelin secretion by long-chain fatty acid through GPR120 in isolated gastric ghrelin cells and mice

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Lu X, Zhao X, Feng J, Liou AP, Anthony S, Pechhold S, Sun Y, Lu H, Wank S. Postprandial inhibition of gastric ghrelin secretion by long-chain fatty acid through GPR120 in isolated gastric ghrelin cells and mice. Am J Physiol Gastrointest Liver Physiol 303: G367–G376, 2012. First published June 7, 2012; doi:10.1152/ajpgi.00541.2011.—Ghrelin is a gastric peptide hormone that controls appetite and energy homeostasis. Plasma ghrelin levels rise before a meal and fall quickly thereafter. Elucidation of the regulation of ghrelin secretion has been hampered by the difficulty of directly interrogating ghrelin cells diffusely scattered within the complex gastric mucosa. Therefore, we generated transgenic mice with ghrelin cell expression of green fluorescent protein (GFP) to enable characterization of ghrelin secretion in a pure population of isolated gastric ghrelin-expressing GFP (Ghr-GFP) cells. Using quantitative RT-PCR and immunofluorescence staining, we detected a high level of expression of the long-chain fatty acid (LCFA) receptor GPR120, while the other LCFA receptor, GPR40, was undetectable. In short-term-cultured pure Ghr-GFP cells, the LCFAFs docosadienoic acid, linolenic acid, and palmitoleic acid significantly suppressed ghrelin secretion. The physiological mechanism of LCFA inhibition on ghrelin secretion was studied in mice. Serum ghrelin levels were transiently suppressed after gastric gavage of LCFA-rich lipid in mice with pylorus ligation, indicating that the ghrelin cell may directly sense increased gastric LCFA derived from ingested intraluminal lipids. Meal-induced increase in gastric mucosal LCFA was assessed by measuring the transcripts of markers for tissue uptake of LCFA, lipoprotein lipase (LPL), fatty acid translocase (CD36), glycosylphosphatidylinositol-anchored HDL-binding protein 1, and nuclear fatty acid receptor peroxisome proliferator-activated receptor-γ. Quantitative RT-PCR studies indicate significantly increased mRNA levels of lipoprotein lipase, glycosylphosphatidylinositol-anchored HDL-binding protein 1, and peroxisome proliferator-activated receptor-γ in postprandial gastric mucosa. These results suggest that meal-related increases in gastric mucosal LCFA interact with GPR120 on ghrelin cells to inhibit ghrelin secretion.

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LCFA inhibition on ghrelin secretion was further investigated in animal studies which suggest that gastric mucosal exposure to LCFA from the lumen and uptake from the circulation inhibit ghrelin secretion. Taken together, these results suggest that meal-related increases in gastric mucosal LCFAAs directly inhibit ghrelin cell secretion through GPR120.

MATERIALS AND METHODS

Materials

DMEM with 4.5 g/l d-glucose, 1-glutamine, and 110 mg/l sodium pyruvate, phenol red-free DMEM, and FBS were obtained from Gibco (Carlsbad, CA); Dulbecco’s PBS (DPBS) and HBSS from Mediatech (Herndon, VA); 16% paraformaldehyde (PFA) and Tissue-Tek OCT compound from Electrical Microscopy Sciences (Hatfield, PA); TRIzol from Invitrogen (Carlsbad, CA); rat tail collagen type I from BD Bioscience (Bedford, MA); Spectra/Mesh nylon filters from Spectrum Laboratories (Miami, FL); docosadienoic acid, linolenic acid (LLA), palmitoleic acid, methyl linolenate, dodecanoic acid, butyric acid (BTA), leptin (Lep), glucagon (Gcg), and Ins from Sigma (St. Louis, MO); and Sst, oxyntomodulin/glucagon-like peptide 1 (GLP), glucose-dependent insulinotropic polypeptide (GIP), octreotide (Oct), and growth hormone (GH) from American Peptide (Sunnyvale, CA).

Ghrelin-GFP Transgene Construction

The vector for constructing ghrelin-GFP transgene was pEGFP-1 (Clontech). To overcome potential positional effects, a 250-bp insulator from the chicken ß-globin locus was introduced into the transgene construct (4). Two copies of chicken ß-globin insulator were amplified by PCR from the vector containing ß-globin insulator (kindly provided by Suming Huang and Gary Felsenfield, Physical Chemistry Section, National Institute of Diabetes and Digestive and Kidney Diseases) and inserted into the plasmid pEGFP-1 at the 5’ and 3’ ends of the enhanced GFP gene between XhoI and SalI and between AflI and AflII, respectively. A second XhoI site was introduced at the end of the 3’ insulator. This new vector was referred to as pInsulator-GFP (Fig. 1A). Early studies demonstrated that up to 5 kb of the 5’-flanking region of the human ghrelin gene from the translational start site contains the necessary regulatory sequences (22). A mouse genomic DNA sequence from –5543 to +18 bp of the ghrelin gene translation start site was amplified by PCR. Primers used for the PCR amplification were Ghre5’-KpnI-s and Ghre3’-SmaI-anti (5’-CATAG-GTACCTGTCAACCAGTGCA-3’ and 5’-GTCCCGGTGTCCTGAAGACAGCATGGTC-3’, respectively). This 5,561-bp PCR amplicon was verified by DNA sequencing. GFP expression was tested by subcloning the ghrelin-GFP transgene into pcDNA 3.1(+) plasmid (Invitrogen) and in vitro transfection in HEK-293 cells. The final 7.6-kbp transgene construct, Pghrelin-GFP, was verified by DNA sequencing. GACAGCATGGTC, respectively). This 5,561-bp PCR amplicon was inserted into the vector pInsulator-GFP in frame with the GFP gene sequences between restriction enzyme KpnI and SmaI sites to generate the transgene construct (Fig. 1B).

Immunofluorescence in Gastric Tissue Sections

Mice were euthanized by CO2 inhalation and perfused with 20 ml of DPBS followed by 30 ml of 4% PFA in DPBS via cardiac perfusion. The whole stomach and 1 cm of proximal duodenum were dissected, cleaned with DPBS, fixed in 4% PFA (3 h to overnight) and incubated in 30% sucrose solution (24 h). Tissues were embedded in Tissue-Tek OCT compound and frozen on dry ice. Cryostat tissue sections (7–9 μm) on positive-charged glass slides were air-dried and stored at 4°C. For immunofluorescence staining, slides were fixed in 4% PFA for 30 min at room temperature, permeabilized in 0.2% Triton X-100 in DPBS for 5 min at room temperature, blocked in 5% FBS and 1% BSA in DPBS for 60 min at room temperature, incubated with primary antiserum for 60 min at room temperature or overnight at 4°C, and detected following incubation with Alexa Fluor-conjugated secondary antiserum for 60 min at room temperature.

Immunofluorescence in Dispersed and Isolated Gastric Cells

Cells dispersed from gastric tissue or collected from FACS were washed once in DPBS, fixed in 4% PFA for 30 min at room temperature, washed once in DPBS, seeded on a positive-charged glass slide (5 μl per slide) and air-dried for 30 min at room temperature. The dried cells were fixed again in 4% PFA for 10 min at room temperature, permeabilized in 0.1% Triton X-100 for 5 s at room temperature, blocked in 1% BSA and 5% FBS in DPBS for 30 min at room temperature, incubated with primary antibody for 60 min at room temperature or overnight at 4°C, and detected following...
incubation with Alexa Fluor-conjugated secondary antibody for 60 min at room temperature.

**Antibodies**

Primary antibody working dilution and source were as follows: rabbit anti-acylated ghrelin (1:500 dilution; YuXiang Sun, Baylor College of Medicine, Houston, TX); rabbit anti-histidine decarboxylase (HDC; 1:1,000 dilution; Alpco, Salem, NH), rabbit anti-Sst and rabbit anti-serotonin (1:100 dilution; Invitrogen), goat anti-Sst (1:1,000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-GFP (1:1,000; Abcam, Cambridge, MA), and rabbit anti-GPR120 (1:2,000–8,000 dilution; catalog no. LS-A2004, Lifespan Biosciences, Seattle, WA). Secondary antibody working dilution and source were as follows: Alexa Fluor 594 goat anti-rat IgG specific for heavy and light chains (H+L), Alexa Fluor 488 chicken anti-goat IgG (H+L), Alexa Fluor 594 donkey anti-goat IgG (H+L), and Alexa Fluor 633 donkey anti-goat IgG (H+L) (1:1,000 dilution; Invitrogen).

**Isolation of Ghr-GFP Cells**

Ghrelin-GFP transgenic mice were euthanized by CO₂ inhalation and flushed with 20 ml of DPBS via cardiac perfusion. The stomach body was dissected, cut open along the lesser curvature, and washed with cold DPBS. Food residues were gently scraped from the surface of the mucosa. Stomach tissues were incubated in 10 ml of DMEM with 2 mg/ml Pronase at 37°C with constant shaking (100 rpm) for 1 h, incubated in 10 ml of fresh Pronase medium for 20 min, and then transferred to 30 ml of phenol red-free DMEM with 10% FBS and shaken to release single cells. Cells were precipitated at 400 g for 5 min at 4°C. The cell pellet was resuspended in phenol red-free DMEM with 10% FBS and filtered through a 20-μm nylon mesh. The dispersed single cells were resuspended in phenol red-free DMEM with 10% FBS at a density of 10⁷ cells/ml for cell sorting. A FACS Aria (BD Biosciences, San Jose, CA) sorter was used to separate Ghr-GFP cells from a mixed population of gastric cells. The excitation wavelength was 488 nm, emission wavelengths were 530 nm (filtration 530/30) and 575 nm (filtration 575/26), and the nozzle size was 100 μm. Single cells were selected by forward scatter (FSC) and side scatter (SSC); then GFP-expressing (GFP) cells were isolated on the basis of their relative fluorescence intensity, and non-GFP-expressing cells were isolated by a gate that excluded cells expressing green fluorescence. Sorted cells were collected into 1 ml of DMEM with 60% FBS on ice.

**Ghrelin Release Assay**

FACS-sorted cells were washed twice, suspended in HBSS supplemented with 20 mM HEPES and 1.2 mM CaCl₂, pH 7.4, seeded as 1,000 cells/well in 100 μl in 96-well round-bottom cell culture plates precoated overnight with 0.1 mg/ml of rat-tail collagen type I, and incubated at 37°C in 5% CO₂. Approximately 99%, 95%, and 90% of cultured cells were viable, as assessed by Trypan blue staining after 1, 2, and 4 h of incubation, respectively. Therefore, the ghrelin secretory responses were measured after 1 h of incubation. Different ligands with various concentrations were added to the reaction buffer in triplicate and incubated for 1 h. The plates were centrifuged at 700 g for 10 min at 4°C, and 25 μl of supernatant were immediately sampled from each well for acylated ghrelin measurement using a Rat Acylated Ghrelin Enzyme Immunoassay Kit (Cayman, Ann Arbor, MI). The secretory response of ghrelin cells to administered ligands was expressed as a percentage of released acylated ghrelin from cells exposed to buffer alone.

**RNA Extraction and Quantitative RT-PCR**

*Gastric tissue. C57BL/6 mice fed standard rodent chow ad libitum or only water for 16 h were euthanized, and the stomach was dissected and cleaned in cold DPBS. Gastric oxyntic mucosa was scraped for RNA extraction and quantitative RT-PCR analysis.

**FACS-sorted cells.** GFP cells and non-GFP cells (1 × 10⁵–1 × 10⁶ cells) collected following FACS were precipitated and dissolved in 0.8–1 ml of TRIZol, and total RNA was extracted according to the manufacturer’s instructions. Synthesis of cDNA from 0.1–1 μg of RNA was accomplished using High-Capacity cDNA Reverse Transcription Kits [Applied Biosystems (ABI), San Jose, CA] followed by quantitative RT-PCR (qRT-PCR) using a StepOnePlus Real-Time PCR System (ABI). Mouse β-actin was used as the housekeeping gene to normalize RNA sample input variability. Gene expression was analyzed using the comparative cycle number at threshold (Ct) method (ABI User Bulletin No. 2). Gene expression was normalized by the constitutively expressed gene β-actin to obtain a ΔCt value. The gene expression relative to β-actin was expressed as 2⁻ΔΔCt and used graphically to demonstrate gene expression.

**Pylorus Ligation and Gastric Gavage**

Twelve 3-mo-old male C57BL/6 mice were fasted for 16 h with access to water. On the following morning, under isoflurane anesthesia, all mice underwent pylorus ligation; then the abdominal wound was closed. Mice with pylorus ligation randomly received no gavage (sham control, n = 4), water gavage (0.5 ml, n = 4), or olive oil gavage (0.5 ml, n = 4). Blood was sampled from the retroorbital sinus for ghrelin measurement prior to pylorosis ligation (~15 min), 15 min after pylorosis ligation but just prior to gastric gavage (0 min), and 30 and 60 min postgavage.

**Data Analysis**

Values are means ± SE. Significant differences between means were analyzed by one- or two-way ANOVA followed by Tukey’s post hoc test and Student’s t-test using GraphPad Prism version 5 software.

**RESULTS**

**Generation of Ghrelin-GFP Transgenic Mice**

A total of 25 litters were generated by pronuclear injection of the ghrelin-GFP transgene (Fig. 1). Two founders with high transgene copy numbers displayed GFP expression along the gastrointestinal epithelium. All the founders and their progeny were viable, healthy, and fertile and exhibited no phenotypic difference compared with their wild-type littermates. GFP cells decreased in density from the stomach to the distal small intestine, with the highest density in the gastric oxyntic mucosa (Fig. 2), where most GFP cells were distributed at the bottom two-thirds of the gastric gland, while some presented near the surface of the gastric mucosa (Fig. 2A, left). There were fewer GFP cells in the gastric antrum (Fig. 2B) and proximal small intestine (Fig. 2C) than in the gastric oxyntic mucosa, and GFP cells were rare in the distal small intestine and colon (data not shown). In the small intestine, the majority of GFP cells were located in the crypts; few were present in the villi (Fig. 2C). Most GFP cells at the gastric oxyntic mucosa were spherical to oval in shape; at the gastric antrum and intestine, GFP cells were teardrop in shape, with thin processes extending to the gut lumen. Since the density, morphology, and distribution of GFP cells between the two founders were indistinguishable, the founder line with the higher transgene copy number was selected and used for subsequent evaluation.
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peptide; and serotonin, an enterochromaffin cell-specific hormone/neurotransmitter. As shown in Fig. 3B, there was no overlap between HDC-IR cells and GFP cells, except a few (<1%) HDC-positive cells showed faint green fluorescence. There were nearly three times more GFP cells than Sst-IR cells. The majority of the Sst cells displayed faint green fluorescence, and >95% of GFP cells with low fluorescence intensity and small irregular shape were Sst-positive (Fig. 3C, arrow). Unlike the other gastric endocrine cells, enterochromaffin cells were predominantly located at the top one-third of the gastric mucosa and did not overlap with GFP cells (Fig. 3D).

Isolation of a Pure Population of Gastric Ghrelin Cells

Cells dispersed from the stomachs of the ghrelin-GFP transgenic mice were a mixed single-cell population. The first attempt to isolate these Ghr-GFP cells on the basis of fluorescence emissions did not allow a clear separation because of the low-level expression of GFP in Sst cells and other gastric mucosa cells. To characterize and separate the Ghr-GFP cells from Sst cells and other non-Ghr-GFP cells by FACS, ghrelin and Sst cells in the mixed-cell population were labeled, respectively, by immunofluorescence with Alexa 630 fluorophore. This allowed clear separation of ghrelin cells from non-ghrelin cells (Fig. 4B1, red) and Sst-IR cells from non-Sst cells (Fig. 4C1, blue). In addition, the ghrelin cells (Fig. 4B2) and the Sst cells (Fig. 4C2) presented different distributions on FSC and SSC. Most ghrelin cells displayed higher fluorescence intensity than the Sst cells (Fig. 4, B3 and C3). Therefore, on the basis of these differences between ghrelin cells and Sst cells, Ghr-GFP cells were first separated from Sst cells by FSC and SSC (Fig. 4D) and subsequently isolated and collected on the basis of fluorescence intensity (Fig. 4E). Success of these two separating steps in achieving a highly enriched Ghr-GFP cell population was demonstrated by 17- and 10-fold greater mRNA expression levels of ghrelin and ghrelin O-acyltransferase, respectively, in Ghr-GFP cells than in Sst-expressing GFP (Sst-GFP) cells. Conversely, Sst gene expression was fourfold higher in Sst-GFP cells than in Ghr-GFP cells (Fig. 4F). Immunostaining confirmed that all isolated Ghr-GFP cells were ghrelin-IR and not Sst-IR (Fig. 4G). However, only 30–40% of presumed Sst-GFP cells were Sst-IR, and 15% were ghrelin-IR (Fig. 4H); the remaining non-ghrelin and non-Sst GFP cells may represent the GFP cells near the surface of the gastric mucosa (Fig. 2A). This pure population of gastric Ghr-GFP cells was subsequently used to study the regulation of ghrelin secretion.

mRNA Expression of Peptide Hormone Receptors in Isolated Gastric Ghr-GFP Cells

Various peptide hormones have been reported to regulate gastric ghrelin secretion (18, 21, 26, 27). To verify whether these peptide hormones have effects on ghrelin secretion, the mRNA expression levels of each receptor for these peptide hormones were measured in isolated pure Ghr-GFP cells. As shown in Fig. 5A, expression of Gcg receptor (GcgR), Ins receptor (InsR), and type 2 Sst receptor (SstR2) was not significantly different between Ghr-GFP and non-GFP cells, and Lep receptors (LepR) were barely detectable in Ghr-GFP cells. However, the Ghr-GFP cells had a significantly greater (12-fold) expression of GIP receptor (P < 0.05) and a 38-fold lower expression of GLP-1 receptor (P < 0.01) than non-GFP

GFP Cells With High Fluorescence Intensity in the Gastric Oxyntic Gland Are Ghr-GFP Cells

To determine whether the GFP cells are ghrelin cells, tissue sections from the stomach were stained for acylated ghrelin. Consistent with published data (6, 24), ghrelin-immunoreactive (IR) cells are located at the lower two-thirds of the gastric oxyntic gland. Nearly all ghrelin-IR cells were GFP-positive. Approximately 90% of GFP cells were ghrelin-IR (Fig. 3A), appearing as spherical to oval shape with high fluorescence intensity. The remaining GFP cells were not ghrelin-IR and displayed an irregular shape with low fluorescence (Fig. 3A, arrows). The GFP cells near the surface of gastric mucosa showed faint fluorescence and were not ghrelin-IR.

To specify the GFP cells that were ghrelin-IR-negative, gastric tissue sections were immunostained for HDC, an enterochromaffin-like cell-specific enzyme; Sst, a D cell-specific enzyme; Sst, a D cell-specific enzyme. The GFP cells near the surface of gastric mucosa showed faint fluorescence and were not ghrelin-IR.
cells. Of the five SstR subtypes, expression of SstR1 ($P < 0.01$) and SstR3 and SstR5 ($P < 0.05$) was significantly higher in Ghr-GFP cells, while expression of SstR4 was significantly lower (16-, 18-, 2.6-, and 225-fold, respectively). GH has been reported to feed back negatively on ghrelin secretion (34); however, expression of the GH receptor in Ghr-GFP cells was not significantly different from that in non-GFP cells.

mRNA Expression of Free Fatty Acid Receptors in Isolated Gastric Ghr-GFP Cells

Intravenous infusion of intralipid increases plasma free fatty acid (FFA) concentration and reduces plasma ghrelin levels (12). Two LCFA receptors, GPR40 and GPR120, and two short-chain fatty acid receptors, GPR41 and GPR43, have been identified (15). To determine whether FFAs directly regulate ghrelin secretion, the mRNA expression levels of FFA receptors in isolated gastric Ghr-GFP cells were determined. GPR40 and GPR41 were undetectable in Ghr-GFP and non-GFP cells. GPR43 and GPR120 were expressed in both cell populations. The expression of GPR43 and GPR120 was threefold greater in Ghr-GFP cells than in non-GFP cells, but GPR120 showed much higher expression levels than GPR43 (Fig. 5B). The expression of GPR120 was confirmed at the protein level by immunofluorescence colocalization of GPR120, with highly expressing GFP cells in presorted and sorted gastric cells as well as gastric tissue sections (Fig. 5C).

Ghrelin Secretion From Isolated Short-Term-Cultured Pure Ghr-GFP Cells

The functional significance of the ghrelin cell surface receptors identified by qRT-PCR was assessed by ghrelin secretion in vitro using acutely isolated cultured gastric Ghr-GFP cells. Consistent with the hormone receptor expression profile, the short-term-cultured Ghr-GFP cells showed no secretory response to Ins (10 nM), Gcg (100 nM), and Lep (10 nM) (Fig. 6A). GH, GIP, and Oxyn, at 10 nM, had no effect on ghrelin release. In contrast, the Sst agonist Oct decreased ghrelin secretion by 10.9 ± 2.1% at 10 nM and 19.9 ± 3.3% at 100 nM (Fig. 6A). The LCFA docosadienoic acid, LLA, and palmitoleic acid, at 2 μM, significantly decreased ghrelin release to
31.5 ± 2.9%, 25.2 ± 3.6%, and 26.2 ± 3.6% of baseline, respectively (Fig. 6B). No effect on ghrelin secretion was observed from methyl linolenate, a pharmacologically inactive form of LLA, the medium-chain fatty acid dodecanoic acid, or the short-chain fatty acids VLA and BTA (Fig. 6B). To ensure consistent secretory competency, the depolarizing effect of 50 mM KCl was used for each preparation of Ghr-GFP cells (Fig. 6C).
Effect of Lipid Gavage on Ghrelin Secretion

To test whether gastric luminal LCFA inhibited gastric ghrelin secretion, pure olive oil, an oleic acid-rich lipid, was introduced into the stomach of wild-type mice by gastric gavage. To avoid the interference of intestinal and postabsorptive LCFA on ghrelin secretion, the passage of stomach lumen content to the intestine was blocked by pylorus ligation. Pylorus ligation induced a sharp drop of serum acylated ghrelin levels to 50% of baseline (preligation) levels (from 598.4 ± 17.7 ng/ml) in 15 min (Fig. 7), after which serum ghrelin levels started to rise. Therefore, gastric gavage was performed 15 min after pylorus ligation (0 time point for gastric gavage). As shown in Fig. 7, gastric gavage of water had no effect on serum ghrelin levels at 30 min (420 ± 52 ng/ml) and 60 min (657.1 ± 59.8 ng/ml) after gavage compared with mice with only pylorus ligation. In contrast, gastric gavage of olive oil caused a delayed recovery of serum ghrelin levels at 30 min (248.2 ± 42.1 ng/ml) compared with sham and water gavage control mice (P < 0.05). At 60 min, ghrelin secretion had recovered to a baseline level comparable to the control groups. This result suggests that gastric luminal lipids cause a transient inhibition of ghrelin secretion.
Increased Prandial Gastric Mucosal Uptake of LCFA

LCFA within the gastric mucosa could also interact directly with the ghrelin cell surface receptor GPR120 and inhibit ghrelin secretion. To identify whether a meal increases gastric mucosal LCFA, gene expression of markers for LCFA uptake, LPL (9, 19), glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1) (2), peroxisome proliferator-activated receptor-γ (PPARγ) (7), and CD36 (11, 13), was measured by qRT-PCR in gastric tissue. As expected, the mRNA levels of mouse gastric mucosal LPL, GPIHBP1, and PPARγ increased significantly in fed mice, while CD36 was slightly, but not significantly, higher (Fig. 8) than in fasted mice. These results suggest that, in the fed state, more LCFA would be taken up into the gastric mucosa, where they could interact with GPR120 to inhibit ghrelin secretion.

DISCUSSION

By generating transgenic mice with ghrelin cell-specific expression of GFP, a pure population of Ghr-GFP cells could be isolated...
and the regulation of ghrelin secretion could be directly characterized. Studies in these isolated Ghr-GFP cells allowed identification of expression of the LCFA receptor GPR120 and LCFA inhibition of ghrelin secretion. Gastric gavage of LCFA-rich lipid to mice with pylorus ligation induced transient reduction of serum ghrelin levels, and feeding significantly increased the gene expression of proteins involved in gastric mucosa uptake of LCFA from circulating triglycerides (TG).

Others have successfully used ghrelin cell-specific transgenic expression of GFP to detect ghrelin cell distribution but have not addressed regulation of ghrelin cell secretion (20). Still others have used transgenic expression of fluorescent proteins to isolate pure populations of enteroendocrine cells, which have a scattered distribution that hinders their characterization, similar to ghrelin cells (31, 33). However, in these studies, the fluorescent-labeled cells could only be characterized within a mixed population of intestinal epithelial cells in short-term culture, which, unlike our study, obscures the ability to distinguish direct from indirect effects.

Because of the orexigenic and energy homeostatic properties of ghrelin, much effort has been focused on the effects of peptide hormones on ghrelin secretion. Published results indicate that Gcg increases ghrelin secretion, while Ins and Lep decrease it (21, 23, 26, 27, 35). We demonstrated that receptors for Gcg and Lep were nearly undetectable in Ghr-GFP cells. As expected, functional studies on short-term-cultured Ghr-GFP cells demonstrated no effects of Glu and Lep on ghrelin secretion. Expression of receptors for Ins, GH, and GLP-1 was lower in Ghr-GFP cells than in non-GFP cells. Consistent with the low receptor expression, these peptide hormones, as well as GIP and Oxyn, had no effect on ghrelin secretion. The inconsistency between the published data and our results indicate that the reported effects of multiple hormones on ghrelin secretion are probably indirect. Among the receptors for enteroendocrine peptide hormones, only SstR1 and SstR3 were highly expressed in Ghr-GFP cells. Their functional expression was confirmed by the significant inhibition of ghrelin secretion from short-term-cultured Ghr-GFP cells by the Sst long-acting analog Oct. Sst is a strong inhibitor for most endocrine cells through endocrine and paracrine effects and is released after ingestion of a meal (3, 8). Sst and its analog inhibit ghrelin secretion demonstrated in our study is consistent with the transient reduction of serum ghrelin levels after a meal (5, 28), the morphologically closed-type ghrelin cells observed in our study as well as by others (6, 24) do not support the proposed direct communication to intraluminal nutrients. Therefore, we investigated whether there is an increase in gastric mucosal LCFA following intestinal absorption of TG in fed mice. Ninety percent of LCFA in plasma are delivered as a component of TG (10). Tissue LCFA, a major energy supply for cell metabolism, are extracted from circulating TG. Uptake of LCFA by individual tissues is regulated by energy utilization under the control of LPL, CD36, GPIHBP1, and PPARy. We hypothesized that the energy requirement for the majority of gastric cells is low in the fasting state and increases with a meal to support gastric secretion and motility necessary for digestion. Consistent with this hypothesis, we found that mouse stomach mucosa LPL levels are significantly higher in fed than fasted mice, suggesting an increased LCFA uptake by gastric mucosa with feeding. Increased LPL activity in the tissue capillary bed enhances lipolysis of TG and increases local LCFA concentration (38). PPARy expression is enhanced by glucose and LCFA, and PPARy activates GPIHBP1 expression. The expression of GPIHBP1, an endothelial cell-binding site for LPL and chylomicrons, is induced by PPARy (7). The increase in stomach mucosa PPARy and GPIHBP1 gene expression with feeding is consistent with upregulation of LPL activity. Taken together, food ingestion increases stomach mucosal LPL activity, resulting in increased LCFA concentration available to the gastric interstitial space, where it may directly interact with GPR120 and inhibit ghrelin secretion. In conclusion, the presence of the Ghr-GFP cell surface LCFA receptor GPR120 and the inhibition of LCFA on ghrelin secretion in vitro indicate that GPR120 may mediate LCFA inhibition on ghrelin secretion. Additionally, the direct action of luminal LCFA and meal-induced increases in transcripts for proteins mediating gastric mucosal uptake of circulating LCFA indicate that GPR120 may play an important role in mediating prandial LCFA inhibition of ghrelin secretion in vivo. These results support the need for future investigation of the therapeutic control of ghrelin-related appetite through the modulation of GPR120 activity.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

X.L. and S.A.W. are responsible for conception and design of the research; X.L., X.Z., J.F., S.A., S.P., Y.S., and H.L. performed the experiments; X.L., A.P.L., and S.A.W. analyzed the data; X.L., and S.A.W. interpreted the results of the experiments; X.L. and S.A.W. prepared the figures; X.L. and S.A.W. drafted the manuscript; X.L., Y.S., and S.A.W. edited and revised the manuscript; X.L. and S.A.W. approved the final version of the manuscript.

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