Ciprofibrate stimulates the gastrin-producing cell by acting luminally on antral PPAR-α

Tom C. Martinsen,1,2 Ingunn Bakke,1 Duan Chen,1 Arne K. Sandvik,1,2 Kolbjørn Zahlsen,3 Trond Aamo,3 and Helge L. Waldum1,2
1Faculty of Medicine, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology and 2Clinical Pharmacology and 3Medical, St. Olav’s Hospital HF, Trondheim University Hospital, Trondheim, Norway

Submitted 9 June 2005; accepted in final form 10 August 2005

Martinsen, Tom C., Ingunn Bakke, Duan Chen, Arne K. Sandvik, Kolbjørn Zahlsen, Trond Aamo, and Helge L. Waldum. Ciprofibrate stimulates the gastrin-producing cell by acting luminally on antral PPAR-α. Am J Physiol Gastrointest Liver Physiol 289: G1052–G1062, 2005. First published August 11, 2005; doi:10.1152/ajpgi.00268.2005.—The lipid-lowering drug ciprofibrate stimulates gastrin-producing cells in the rat stomach without lowering gastric acidity. Although suggested to be a luminal action on antral peroxisome proliferator-activated receptor-α (PPAR-α), the mechanism is still not fully elucidated. Gastric bypass was surgically prepared in male Sprague-Dawley rats. Gastric-bypassed and sham-operated rats were either given ciprofibrate (50 mg·kg⁻¹·day⁻¹ in methocel) or vehicle alone for 7 wk. PPAR-α knockout (KO) and wild-type (WT) mice were either given ciprofibrate (500 mg·kg⁻¹·day⁻¹ in methocel) or vehicle alone for 2 wk. The concentration of gastrin in blood was analyzed. Antral G cell density and gastrin mRNA abundance were determined by using immunostaining and Northern blot analysis. Ciprofibrate did not raise plasma gastrin or G cell density in gastric-bypassed rats, although the gastrin mRNA level was slightly increased. In contrast, ciprofibrate induced hypergastrinemia, a 50% increase in G cell density, and a threefold increase in gastrin mRNA in sham-operated rats. In PPAR-α KO mice, ciprofibrate did not raise G cell density or the gastrin mRNA level. The serum gastrin level was reduced by ciprofibrate. In WT mice, ciprofibrate induced hypergastrinemia, a doubling of G cell density, and a threefold increase in gastrin mRNA. Comparing animals dosed with vehicle only, PPAR-α KO mice had higher serum gastrin concentration than WT mice. We conclude that the main effects of ciprofibrate on G cells are mediated from the antrum lumen, and the mechanism is dependent on PPAR-α. The results indicate that PPAR-α may have a role in the physiological regulation of gastrin release.

Peroxisome proliferator-activated receptor-α; hypergastrinemia; gastric bypass; peroxisome proliferator-activated receptor-α knockout mice

IN 1905, EDRINKS (19) postulated the presence of a humoral gastric acid secretagogue being released from the antral mucosa. This was confirmed by Gregory et al. (24) in 1966, who identified this secretagogue as gastrin. Gastrin is still the only known gut hormone released from the stomach that mediates gastric acid secretion. The secretagogue effect of gastrin is mediated by the gastrin receptor on the histamine-containing ECL cells (25, 56, 72). When activated, the ECL cells respond with increased production (55) and release of histamine (56), which stimulates acid secretion from parietal cells by acting on histamine-2 receptors (38, 39, 71). In addition, gastrin is acknowledged to be the most important growth factor in the oxyntic mucosa (26, 73, 77). The trophic effect of gastrin is also mediated by the gastrin receptor (77). Consequently, the ECL cell constitutes the main target of gastrin in the regulation of both function and growth of the oxyntic mucosa (7). Long-term hypergastrinemia leads to hyperplasia of the oxyntic mucosa, and especially the ECL cell (2), in both rodents (28, 51) and humans (13, 61). In several animal models, hypergastrinemia has induced ECL cell-derived gastric tumors (28, 42, 51, 75). Moreover, hypergastrinemic states such as atrophic gastritis and Zollinger-Ellison syndrome are associated with the development of gastric tumors (5, 8, 21, 30, 32, 34, 61). There is an ongoing debate concerning the role of hypergastrinemia in human gastric carcinogenesis (52, 69).

Gastrin-producing cells (G cells) constitute one of the major populations of neuroendocrine cells in the antral mucosa of the stomach. Gastrin is released from secretory granules at the basolateral membrane, which is close to mucosal blood vessels, reaching the oxyntic mucosa by the systemic circulation (38, 39). Gastrin release is primarily regulated by luminal stimuli. The G cell is an open-type endocrine cell organized with microvilli on the apical membrane on the luminal surface, being able to detect and sample luminal contents continuously (38, 39). Chemical constituents of the food, especially amino acids, dietary amines (15), calcium (4, 9), and gastric hypochlorhydria, provide the strongest stimulation of gastrin release (38, 39). H⁺ has an inhibitory effect on G cell activity at pH <4, and an increase in gastric pH leads to a decrease in G cell inhibition and an increase in gastrin release (74). In addition to luminal factors, somatostatin from adjacent D cells (38, 39) as well as both cholinergic and noncholinergic intramural neurons (57) modulate G cell function.

Peroxisome proliferators (PP) are a large group of heterogenous compounds that regulate gene expression via peroxisome proliferator-activated receptors (PPARs). PPARs have become a topic of intensive research (68) since the discovery of these nuclear steroid receptors by Issemann and Green (31) in 1990. These receptors are found in all organs but are most abundant in the liver and adipose tissue. Until now, three isoforms of PPARs have been demonstrated (α, β, and γ; see Ref. 60), and several PPs are already available for clinical use (22, 64).

Stimulators of PPAR-α have a well-known tumorigenic effect on the rodent liver (53). The exact mechanism is not known, but it seems to be nongenotoxic (48).

The effects of PP on the stomach became an issue of interest because of the findings of ECLomas in the oxyntic mucosa of...
rats after long-term dosing with PPAR-α agonists such as clofibrate and ciprofibrate (63). The animals were found to be hypergastrinemic, and consequently the tumorigenesis was initially attributed to hypergastrinemia secondary to a suggested acid inhibitory effect of these fibers (16, 17). This was analogous to rodents developing ECLomas during hypergastrinemia induced by drugs inhibiting gastric acid secretion (28, 51). However, the reported inhibition of acid secretion of these PPAR-α agonists was only moderate in contrast to the profound inhibition needed to induce similar ECL cell changes in other models of hypergastrinemia (43). A reexamination of the effect on acid secretion in rat showed that ciprofibrate induced a dose-dependent hypergastrinemia without reducing gastric acid secretion at all (43). Moreover, the hypergastrinemic effect developed gradually after 2–3 wk of dosing (27), and combination with a proton pump inhibitor revealed a potentiating hypergastrinemic effect (27). Furthermore, ciprofibrate-induced hypergastrinemia is not reversed by the somatostatin analog octreotide (3), in contrast to hypergastrinemia caused by inhibition of acid secretion (45). All these findings strongly indicate that the fibers stimulate the G cell directly.

The mechanism of PP-induced G cell stimulation is so far not studied thoroughly. The stimulation has been suspected to be mediated by PPAR-α, because several PPAR-α agonists, in contrast to a PPAR-γ agonist (troglitazone), induce hypergastrinemia (1). The demonstration of PPAR-α immunoreactivity in G cells also supports this conclusion (1). Normally, there is a reciprocal relationship between the activity of the antral G and D cells (6, 76). During ciprofibrate dosing, we found a concomitant increase in gastrin and somatostatin mRNA abundance in the antral mucosa (3, 70). To our knowledge, this had not been reported previously. In contrast, the abundance of somatostatin mRNA in the oxyntic mucosa was decreased during ciprofibrate dosing (70). Knowing that antral D cells are open and oxyntic D cells are closed, it seems reasonable to suggest that ciprofibrate activates endocrine cells of the open type by a direct luminal effect (70). Induction of the peroxisomal enzyme CoA oxidase (ACO) is one of the most frequently used markers of PP action and PPAR-α stimulation (49, 67). In ciprofibrate-dosed rats, we found an increased abundance of ACO mRNA in the antrum, whereas the expression of this gene was unchanged in the oxyntic mucosa (1). This also suggests a local effect of ciprofibrate in the antrum.

This study examines the mechanism of PP-induced G cell stimulation in surgically modified (oesophago-duodenostomy) rats and expands our previous observations to another species [wild-type (WT) and PPAR-α knockout (KO) mice].

MATERIALS AND METHODS

Test substance. Ciprofibrate [2-[4(6–2,2 dichlorocroplpyl)phenolxyl]-2-methyl propanoic acid; Sanofi-Synthelabo, Alnwick, UK] was used as a PPAR-α-specific agonist. It was suspended in 2% methocel (methylcellulose, M7140; Sigma, St. Louis, MO) at a concentration of 20 mg/ml.

Animals and animal management. The study was approved by the Animal Welfare Committee at the University Hospital of Trondheim.

Rats. Male Sprague-Dawley rats (220 g body wt; Møllegaard, Skensved, Denmark) were housed in wire-top cages (5 in each cage) at 20°C with 40–60% relative humidity and a 12:12-h light-dark cycle. The standard rat food pellets (B&K Universal) and tap water were provided ad libitum. Before blood sampling, the animals were anesthetized with a subcutaneous injection of 0.02 ml/kg of a solution containing 2.5 mg/ml fluanison, 0.05 mg/ml fenital (Tamson Animal Health, Buckinghamshire, UK), and 1.25 mg/ml midazolam (Alpharma, Oslo, Norway). The animals were freely fed before surgery and blood sampling. Animals subjected to either gastric bypass or sham operation were anaesthetized with 0.03 ml/kg of the same anaesthetic solution. The gastric bypass was created by an anastomosis of the lower esophagus to the first segment of duodenum end to side, leaving the stomach connected to the duodenum but not to the esophagus. The sham operation included laparotomy through a midline abdominal incision followed by gentle manipulation of the viscera. The animals were killed by puncture of great vessels close to the heart under anesthesia.

Mice. PPAR-α homozygous KO mice (PPAR-α−/−) have been described previously (37). An equal number of males and females weighing 20–25 g were used. Age-, sex-, and weight-matched WT mice homozygous for the receptor gene (PPAR-α+/+) were used as controls. The PPAR-α−/− and PPAR-α+/+ lines were purebred on a sv129 background. All mice were a generous gift from Frank J. Gonzalez (National Cancer Institute, Bethesda, MD). The mice were housed in wire-top cages (10 in each cage) at 20°C with 40–60% relative humidity and a 12:12-h light-dark cycle. The standard mice food pellets (B&K Universal) and tap water were provided ad libitum. Before death, the mice were anesthetized by subcutaneous injection of 0.02 ml/kg of the solution described above. The mice were killed by puncture of great vessels close to the heart under anesthesia.

Study Design

Rats. Two groups of gastric-bypassed (n = 8 in each group) and two groups of sham-operated (n = 8 in each group) rats were either given 50 mg/kg ciprofibrate in methocel, a dose previously shown to induce hypergastrinemia in rats (3, 43), or 0.5 ml vehicle alone by daily gastric intubation for 7 wk. Unfortunately, among the gastric-bypassed animals, one individual in the ciprofibrate group and three in the methocel group died during the first wk of the study. Consequently, there were seven animals in the ciprofibrate group and five animals in the methocel group at 4 wk. Between 4 and 7 wk, some more gastric-bypassed animals died, and at termination of the study (after 7 wk of dosing) there were four animals in each group. Blood was collected from the femoral vein at the start of the study, after 4 wk (after 7 wk of dosing) there were four animals in each group. Blood more gastric-bypassed animals died, and at termination of the study (after 7 wk of dosing) there were four animals in each group. Blood was collected from the femoral vein at the start of the study, after 4 wk, and after 7 wk. Plasma was frozen at −20°C for later determination of gastrin concentration by RIA (33) and ciprofibrate concentration by HPLC. At death, the stomachs were immediately removed, opened along the greater curvature, and rinsed in saline. Samples were collected from the antral mucosa at a standardized location and fixed in 4% buffered formaldehyde or homogenized for RNA extraction. The homogenates were frozen at −80°C for later processing.

Mice. Two groups of PPAR-α KO mice and WT mice were either given 500 mg/kg ciprofibrate in methocel (n = 18 in each group) or 0.2 ml of the vehicle alone (n = 12 in each group) by daily gastric intubation for 2 wk. Ciprofibrate-induced hypergastrinemia was previously found to be only transient in mice (18); however, the dose used (20 mg/kg−1day−1) has only been the same as used in rats. Preliminary studies in our laboratory have shown that higher doses of ciprofibrate are needed to induce hypergastrinemia in mice compared with rats. With the use of 500 mg-kg−1day−1 ciprofibrate, mice became hypergastrinemic after only 2 wk. At death, blood was collected from the inferior vena cava. Serum was frozen at −20°C for later determination of gastrin concentration by RIA and ciprofibrate concentration by HPLC. The stomachs were immediately removed, opened along the greater curvature, and rinsed in saline. Samples were collected from the antral mucosa at a standardized location and fixed in 4% buffered formaldehyde or homogenized for RNA extraction. The homogenates were frozen at −80°C for later processing. The liver was removed and weighed.
**Immunohistochemistry**

The fixed biopsies were dehydrated in ethanol, embedded in paraffin, and cut perpendicularly to the mucosal surface in 5-μm-thick sections (Leica 2055 Autocut). All sections were dewaxed, rehydrated, and treated with 3% H₂O₂ for 10 min to block endogenous peroxidase activity. G cells were stained by incubation with rabbit antihuman gastrin (A0568, dilution 1:700; Dako) for 2 h at room temperature followed by the EnVision-horseradish peroxidase technique (K5007; Dako). The immunoreactivity was detected using the AEC peroxidase kit (SK4200; Vector Laboratories, Burlingame, CA). As a negative control, the primary antiserum was substituted with diluted nonimmune rabbit normal serum (X0936; Dako). All sections were counterstained with hematoxylin.

G cell density. The G cell density in antral gastric glands was assessed by counting the number of nucleated gastrin immunoreactive cells per millimeter mucosa. Sections from five different animals from each group were used. In each section, five randomly selected areas of the same width as the grid (250 μm) were counted.

**Northern Blot Analysis**

Biopsies for RNA analysis were homogenized (Ultra-Turrax homogenizer; Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) in a denaturing buffer (1 ml/100 mg tissue) containing guanidinium isothiocyanate (4 M), sodium acetate (25 mM, pH 6.0), and β-mercaptoethanol [0.84% (vol/vol)]. Total RNA was isolated by ultracentrifugation through a cesium chloride (5.7 M) cushion and precipitated with ethanol. The probes for somatostatin, gastrin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and 28S rRNA, and the mRNA abundances were normalized to 18S ribosomal RNA. Sizes of the different mRNAs were estimated with reference to 18S and 28S rRNA, and the mRNA abundances were normalized to 18S values. GAPDH abundance was used as control. The hybridization probes, all generated from rat cDNA, showed a 90–97% homology to the mRNA measured on Northern blot were as expected for the mouse. The probes were purified on NICK columns (Pharmacia Biotech, Uppsala, Sweden).

Northern blots were prepared by electrophoresing total RNA (20 μg/well) in 1% agarose-formaldehyde gels, electroblotting on nylon membranes (Roche, Mannheim, Germany), and cross-linking by ultraviolet irradiation. The membranes were prehybridized for 4 h at 65°C in 5× standard saline phosphate EDTA (SSPE) buffer [1× SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA (pH 7.4)] with 50% formamide, 5°C Denhardt’s solution (0.1% BSA, polyvinylpyrrolidone, and Ficoll 400), 0.5% SDS, and sonicated salmon sperm DNA. The [32P]cRNA probe (2 × 10⁶ counts·min⁻¹·ml⁻¹) was added for overnight hybridization at 65°C. The blots were washed two times in 2× SSPE with 0.1% SDS for 20 min at room temperature and one time in 0.1× SSPE with 0.1% SDS at 65°C, exposed to a storage phosphor screen for 24 h, scanned on a PhosphorImager (LAS-1800II; FujiFilm, Kanawaga, Japan), and quantified using ImageGauge software (version 3.4α, Science Lab 99; Fuji Photo Film). Stripping between hybridizations was done with boiling 0.1% SDS. The [32P]cRNA probe (2 × 10⁶ counts·min⁻¹·ml⁻¹) was added for overnight hybridization at 65°C. The blots were washed two times in 2× SSPE with 0.1% SDS for 20 min at room temperature and one time in 0.1× SSPE with 0.1% SDS at 65°C, exposed to a storage phosphor screen for 24 h, scanned on a PhosphorImager (LAS-1800II; FujiFilm, Kanawaga, Japan), and quantified using ImageGauge software (version 3.4α, Science Lab 99; Fuji Photo Film). Stripping between hybridizations was done with boiling 0.1% SDS.

**Statistics**

One-way ANOVA with Bonferroni’s posttest was used to statistically evaluate three or more groups, whereas an unpaired t-test was used to compare two groups. For all evaluations, P > 0.05 was considered nonsignificant. All values are given as means ± SE.

**RESULTS**

**Plasma/Serum Concentrations of Gastrin**

Rats. After 7 wk, ciprofibrate did not raise plasma gastrin concentration significantly in gastric-bypassed rats compared with vehicle (16.0 ± 3.2 vs. 10.0 ± 0.3 pmol/l, P = 0.089). In contrast, in sham-operated rats, ciprofibrate induced more than a twofold increase compared vehicle (51.8 ± 8.7 vs. 23.4 ± 2.1 pmol/l, P < 0.05). Sham-operated animals dosed with ciprofibrate had close to three times the plasma gastrin concentration found in gastric-bypassed animals dosed with ciprofibrate (P < 0.01). When animals dosed with vehicle only were compared, the gastric bypass operation induced a >50% decrease in the plasma gastrin concentration (P < 0.05; Fig. 1A).

After 4 wk, ciprofibrate did not raise plasma gastrin concentration in gastric bypassed rats compared with vehicle (13.3 ± 1.0 vs. 10.9 ± 1.7 pmol/l, P = 0.23). The plasma gastrin concentrations in the other groups of rats were practically exactly the same differences when different groups of rats were compared (data not shown).

Mice. In PPAR-α KO mice, ciprofibrate induced a decrease in serum gastrin concentration compared with vehicle (17.5 ±
1.9 vs. 27.4 ± 3.5 pmol/l, *P < 0.05). In contrast, in WT mice, ciprofibrate induced a twofold increase in serum gastrin concentration compared with vehicle (31.2 ± 2.8 vs. 15.3 ± 1.9 pmol/l, *P < 0.05). WT mice dosed with ciprofibrate had nearly two times the serum gastrin concentration found in PPAR-α KO mice dosed with ciprofibrate (*P < 0.001). When animals dosed with vehicle only were compared, PPAR-α KO mice had higher serum gastrin concentrations than WT mice (*P < 0.05; Fig. 2A).

**Plasma/Serum Concentrations of Ciprofibrate**

*Rats*. After 7 wk, the concentration in bypassed rats was higher than in sham-operated rats (2,276 ± 240 vs. 1,443 ± 96 μmol/l, *P < 0.01). After 4 wk, there was no difference in the plasma concentration of ciprofibrate in bypassed and sham-operated rat dosing (1,976 ± 179 vs. 1,850 ± 112 μmol/l).

*Mice*. There was no difference in serum concentration of ciprofibrate in PPAR-α KO mice and WT mice (1,510 ± 73 vs. 1,763 ± 152 μmol/l).

**Antral G Cell Density**

*Rats*. In gastric-bypassed rats, ciprofibrate did not raise antral G cell density compared with vehicle (7.7 ± 0.6 vs. 8.3 ± 0.5 no. of G cells/mm mucosa length). In contrast, in sham-operated rats, ciprofibrate induced an ~50% increase in antral G cell density compared with vehicle (14.8 ± 0.6 vs. 9.5 ± 1.0 no. of G cells/mm mucosa length, *P < 0.001). The antral G cell density in ciprofibrate-dosed sham-operated rats was twofold the antral G cell density found in ciprofibrate-dosed gastric-bypassed animals (*P < 0.001). When animals dosed with vehicle only were compared, there was no difference in antral G cell density (Fig. 1B).

*Mice*. In PPAR-α KO mice, ciprofibrate did not increase antral G cell density compared with vehicle (5.8 ± 0.3 vs. 5.8 ± 0.3 no. of G cells/mm mucosa length). In contrast, in WT mice, ciprofibrate induced nearly a twofold increase in antral G cell density compared with vehicle (11.5 ± 0.6 vs. 6.5 ± 0.4 no. of G cells/mm mucosa length, *P < 0.001; Fig. 2B).

WT mice dosed with ciprofibrate had nearly two times the antral G cell density found in PPAR-α KO mice dosed with ciprofibrate (*P < 0.001). There was no difference in antral G cell density between PPAR-α KO mice and WT mice dosed with vehicle.

**Antral Gastrin and Somatostatin mRNA Expression**

*Rats*. In gastric-bypassed rats, ciprofibrate induced a significant increase in the antral abundance of gastrin mRNA (Fig. 3A) compared with vehicle (95 ± 23 vs. 36 ± 8% of control; sham-operated rats given methocel, *P < 0.05). In sham-operated rats, ciprofibrate induced nearly a threefold increase compared with vehicle (274 ± 72 vs. 100 ± 20% of control, *P < 0.05). Sham-operated animals dosed with ciprofibrate had close to three times the antral abundance of gastrin mRNA found in gastric-bypassed animals dosed with ciprofibrate (*P < 0.05). When animals dosed with vehicle only were compared, the bypass operation induced a >50% decrease in the antral gastrin mRNA abundance (*P < 0.05).

No statistically significant differences between the groups in antral abundance of somatostatin mRNA (Fig. 3B) were found, but there was a trend toward increased abundance in sham-operated rats receiving ciprofibrate (142 ± 18% of control) compared with vehicle dosing (*P = 0.057) and compared with bypass-operated rats dosed with ciprofibrate (93 ± 9% of control, *P = 0.089). The antral abundance of GAPDH mRNA was unchanged in all groups.

*Mice*. In PPAR-α KO mice, ciprofibrate did not increase the antral abundance of gastrin mRNA (Fig. 4A) compared with vehicle (124 ± 19 vs. 110 ± 12% of control). In contrast, in WT mice, ciprofibrate induced more than a threefold increase in the antral abundance of gastrin mRNA compared with vehicle (333 ± 79 vs. 100 ± 8% of control, *P < 0.01). WT mice dosed with ciprofibrate had nearly three times the antral abundance of gastrin mRNA found in PPAR-α KO mice dosed with ciprofibrate (*P < 0.01). There was no difference in antral abundance of gastrin mRNA between PPAR-α KO mice and WT mice dosed with vehicle.

No statistically significant change in antral abundance of somatostatin mRNA (Fig. 4B) was found, but in WT mice there was a trend toward increased abundance after ciprofibrate dosing (151 ± 26% of control, *P = 0.074). When KO mice and WT mice dosed with vehicle were compared, the level of somatostatin mRNA seemed to be generally higher in KO mice (159 ± 28% of control, *P = 0.087), but there were no changes.
in the level after dosing with ciprofibrate (138 ± 35% of control). The antral abundance of GAPDH mRNA was unchanged in all groups.

Liver Weights in Mice

In PPAR-α KO mice, ciprofibrate had no effect on liver weights compared with vehicle (0.90 ± 0.03 vs. 0.70 ± 0.04 g). In contrast, in WT mice, ciprofibrate induced more than a fourfold increase in liver weight (0.80 ± 0.03 vs. 3.50 ± 0.05 g, P < 0.001). There was no difference in liver weights between PPAR-α KO mice and WT mice dosed with vehicle.

DISCUSSION

Studies have shown that ciprofibrate induces transcription of gastrin mRNA, hypergastrinemia, and hyperplasia of antral G cells (3, 27, 43, 70). The effect is not mediated by reduced gastric acidity (43) but is potentiated by administration of the proton pump inhibitor omeprazole (27). Furthermore, G cell hyperfunction is not reversed by the normally suppressive somatostatin analog octreotide, indicating a unique and direct effect of ciprofibrate on the G cell (3) independent of gastric acidity and somatostatin. Continuous dosing of ciprofibrate for 2 wk induces significant hypergastrinemia in rats (27), and the effect is not restricted to a particular rat strain or gender (70). The mechanism by which ciprofibrate stimulates antral G cells in the rat has so far been unknown.

Effect of Ciprofibrate on the Antral G cell: a Local Mechanism on an Open Cell

The effect of ciprofibrate on the G cell could in theory be mediated systemically, locally in the antrum, or be a combination of these. The gastric bypass operation as described here excludes the local effect of ciprofibrate in the stomach lumen. Our results clearly demonstrate that the effect of ciprofibrate on antral G cells in gastric-bypassed rats is nearly eliminated. First, in contrast to the sham-operated animals, ciprofibrate had no effect on plasma gastrin concentration in gastric-bypassed animals at 4 wk. At 7 wk, the number of animals was reduced because of mortality, and there was a slight tendency (not significant) to an increase in plasma gastrin level among the gastric-bypassed rats. This is in concordance with the increased abundance of gastrin mRNA found in the same group of animals. Therefore, although not significant, a minor systemic effect on the G cell cannot be excluded.

Fig. 3. Mean antral gastrin (A) and somatostatin (B) mRNA abundances ± SE in sham-operated and gastric-bypassed rats given either ciprofibrate (50 mg/kg) or methocel (vehicle) by daily intubations for 7 wk, expressed as %values in control animals (sham-operated rats given methocel). *P < 0.05.

Fig. 4. Mean antral gastrin (A) and somatostatin (B) mRNA abundances ± SE in WT and PPAR-α KO mice given either ciprofibrate (500 mg/kg) or methocel (vehicle) by daily intubations for 2 wk, expressed as %values in control animals (WT mice given methocel). **P < 0.01.
Second, ciprofibrate induced no increase in density of antral gastrin immunoreactive cells in gastric-bypassed animals. In sham-operated animals dosed with ciprofibrate, there was a 50% increase in the number of immunoreactive G cells per millimeter mucosa. This level of hyperplasia is quite similar to observations in previous rat studies with ciprofibrate (3, 70) and in studies with inhibitors of gastric acid secretion (29, 65). No further increase in G cell density is found in long-term studies (10); thus, close to a doubling of G cell density seems to be the maximal level of G cell hyperplasia independent of cause.

Previous studies show that ciprofibrate induces a concomitant increase in gastrin and somatostatin mRNA abundance in the antral mucosa (3, 70). Normally, an increase in D cell activity can be seen in response to increased gastric acidity (6). However, an increase in somatostatin gene expression is also seen when combining ciprofibrate with the proton pump inhibitor omeprazole (27). This simultaneous stimulation of antral G and D cells is in contrast to their normal reciprocal relationship (6, 76) in which the D cells provide a paracrine tonic inhibition of gastrin release from G cells (6, 20). In addition, G cell gastrin secretion may act to restore somatostatin release from D cells via gastrin receptors on the D cells (36). The stimulatory effect of ciprofibrate on D cells seems to be restricted to the antrum because a decrease in somatostatin mRNA is demonstrated in oxyntic mucosa (3, 70). Because antral D cells are open and oxyntic D cells are closed, it is reasonable to suggest that ciprofibrate activates endocrine cells of the open type from the lumen and not via the systemic circulation. In this study, we found a 42% increase (P = 0.057) in antral somatostatin gene expression during ciprofibrate dosing in sham animals. The antral somatostatin gene expression in gastric-bypassed animals was unchanged, which further supports the theory of a local effect of ciprofibrate on open D cells in the antrum. Similar antrum-specific effects of ciprofibrate have also been shown for peroxisomal enzyme ACO (1) and for PPAR-α (1), supporting the theory of a local effect in the antrum.

The plasma gastrin concentration and the antral abundance of gastrin mRNA in animals given vehicle only were lower in gastric-bypassed rats compared with sham animals. The G cell is an open cell thought to sample luminal content and to respond with increased gastrin expression and secretion. In gastric-bypassed animals, the antral gastric glands, in which G cells are located, are not exposed to food. Consequently, there is no normal luminal stimulation of the G cells. Gastric bypass has been used as treatment of obesity. In these patients, both fasting and postprandial gastrin were lowered postoperatively (44, 46, 58, 59), which is in concordance with lowered G cell activity found in gastric-bypassed rats.

After 4 wk, the plasma concentration of ciprofibrate was equal in gastric-bypassed and sham animals, reflecting that the substance is absorbed equally in the two groups of animals. Because of an unexplained cause, the plasma concentration of ciprofibrate at death was lower in the sham animals compared with the gastric-bypassed animals. This may be explained by gastric destruction. However, despite a higher plasma concentration of ciprofibrate in the gastric-bypassed animals at death, the plasma gastrin concentration was not increased. This further supports that the systemic effect of ciprofibrate on the G cell is insignificant.

In mice, the serum concentrations of ciprofibrate at death were equal in both PPAR-α KO and WT animals, reflecting that the substance is absorbed equally in the two groups.

**Hypergastrinemia Induced by PPAR-α Agonists, Not Only in Rats**

The hypergastrinemia induced by fibrates has so far only been consistently observed in rats. Indeed, Eason et al. (18) reported a transient hypergastrinemia in mice, whereas there was no effect on serum gastrin in marmosets. Accordingly, the histological changes in gastric mucosa have only been shown in rats. However, in previous studies, the same dose per kilogram was used in all species examined. In the present study, it is clearly demonstrated that the G cell-stimulating effect of ciprofibrate is not restricted to rats. By increasing the dose, the mice become markedly hypergastrinemic, emphasizing interspecies variation in pharmacokinetics. Consequently, the G cell-stimulating effects of PPAR-α agonists are not restricted to the rat and possibly may also be found in humans. The recommended daily dose of ciprofibrate in humans to treat hyperlipidemia is usually 100 mg, which is ~30-fold less than the dose shown to induce hypergastrinemia in rats in this study. However, given the variation in responsiveness in mice and rats, it cannot be excluded that a similar G cell-stimulating effect could be found in patients using clinical doses of ciprofibrate.

**PPAR-α in Regulation of Gastrin Release**

Because ciprofibrate is regarded as one of the most specific PPAR-α agonists, its G cell-stimulatory effect has been suspected to be PPAR-α dependent. In recent studies, this suggestion has been substantiated by several findings. First, another specific nonfibrate PPAR-α agonist, WY-14643, was also found to induce hypergastrinemia (1), whereas the PPAR-γ agonist troglitazone failed to do so (1). Second, in rats, ciprofibrate has been shown to increase the antral abundance of mRNA of the peroxisomal enzyme ACO (1), which is the most frequently used marker of PPAR-α stimulation (49, 67). Third, PPAR-α immunoreactivity has been demonstrated in G cells (1), supporting a role of this receptor in G cell stimulation.

The impact of PPAR-α in ciprofibrate-induced G cell stimulation is clearly demonstrated by the use of PPAR-α KO mice. As in rats, the serum gastrin concentration, the antral G cell density, and the gastrin mRNA abundance increased markedly in ciprofibrate-dosed WT mice during the study. It was expected that the level of serum gastrin would remain unaffected in ciprofibrate-dosed PPAR-α KO mice. However, ciprofibrate induced a significant decrease in serum gastrin level in these mice. This indicates that, in the absence of PPAR-α, ciprofibrate may have other effects on G cells. As noted recently, there are precedents for PPAR agonists acting through PPAR-independent pathways (50). Concerning the antral density of gastrin-immunoreactive cells and gastrin mRNA abundance, ciprofibrate induced no changes in the PPAR-α KO animals in contrast to WT animals.

Another striking finding was that, in mice dosed with vehicle only, there was a significant difference in the serum gastrin concentration between WT and KO mice, being higher in the PPAR-α KO animals. This finding is probably not because of
methocel, since unpublished results from our laboratory confirm that serum gastrin level in PPAR-α KO mice not dosed with methocel is higher than in WT mice. Knowing the hypergastrinemic effect of ciprofibrate acting on PPAR-α, this finding was unexpected. There were no differences in antral density of G cells or antral gastrin mRNA abundance between methocel-dosed PPAR-α KO and WT mice. Thus, according to these results, the lack of PPAR-α seems to influence gastrin release without affecting G cell density or gastrin gene expression. An alternative explanation may be that PPAR-α influences enzymes required for progastrin processing. This remains to be settled.

As a control of the KO model, the liver weights were measured at death, showing more than a threefold increase in WT mice dosed with ciprofibrate. No gain in liver weights was found in KO mice, confirming the absence of the well-known trophic effect on the liver induced by PPAR-α agonists.

Ciprofibrate is completely absorbed in the gastrointestinal tract (14). In plasma, it is bound to albumin, and it is eliminated by the kidneys (14). It acts by binding to PPAR-α, which leads to a heterodimerization with an activated 9-cis retinoic acid receptor (41). Subsequently, the ligand receptor complex acts as a transcription factor by binding to DNA (40). PPAR-α is a member of the so-called nuclear steroid hormone receptor family (31); thus ciprofibrate has to pass the luminal membrane of the open G and D cells to reach its target located in the nucleus. Being hydrophobic at low pH, it most probably crosses the cell membrane, reaching PPAR-α passively. Thus the highest intracellular concentration of ciprofibrate in G cells is achieved by local access from the antral lumen rather than from the systemic circulation, which is the most reasonable explanation why the local effect is predominant in G cell stimulation.

Although being an orphan receptor (41), numerous substances have been found to activate PPAR-α. Like the other PPARs, the ligand-binding domain of the α-isofrom consists of a hydrophobic pocket three times larger than those found in other nuclear receptors (47). This gives rise to distinct features reflected by the broad diversity of its ligands. The ligands of the PPARs have relatively low affinity toward their receptors, and some ligands may bind to more than one of the receptor isoforms. These characteristics of the ligand-binding domain, together with a complex process of transcript regulation after receptor activation, make this regulation of gene expression rather complicated (12, 62). Taking into consideration the difference in serum gastrin level in vehicle-dosed PPAR-α KO and WT animals, it seems reasonable to assume that PPAR-α could have a role in the physiological regulation of gastrin release. Located in open cells in the stomach, this receptor is continuously exposed to a variety of substances in the food with potential ligand-binding qualities.

Ciprofibrate is one of several fibrates used in clinical medicine for treatment of dyslipidemia. In spite of the knowledge of ciprofibrate-induced hypergastrinemia in rodents, an effect shown to be potentiated by proton pump inhibitors, there is no relevant human study concerning this issue. Taking into consideration the association between hypergastrinemic states and gastric neoplasms, there is a need for long-term studies also in humans. The only human study on this subject showed an increase in 24-h intragastric acidity after oral administration of clofibrate (23). There was no effect on serum gastrin during the study. From this 24-h study, the authors concluded that human gastrin-induced ECL cell proliferation is unlikely with this drug. Knowing the time needed to induce hypergastrinemia in rats (27), this conclusion seems inadequate. Elevated gastrin concentrations are also suggested to be a risk factor for colorectal cancer (66), and circulating concentrations of nonamidated gastrin precursors are increased in patients with such disease (11). Recently, several PPAR-α agonists were shown to stimulate progastrin production in a human colorectal cell line (LIM 1899; see Ref. 35).

In conclusion (Fig. 5), with the use of gastric-bypassed rats, the main stimulatory effects of ciprofibrate on G cells are found to be mediated locally in the antrum. With the use of PPAR-α

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Fig. 5. Mechanism of ciprofibrate-induced antral G cell stimulation; a local effect on an open cell by activating nuclear PPAR-α; stimulation (+) and inhibition (−) of gastrin release.
KO mice, the main stimulatory effects of ciprofibrate on G cells are found to be mediated by PPAR-α activation.

ACKNOWLEDGMENTS
We thank Bjorn Munkvold, Britt Schulze, and Anne Kristensen for technical assistance.

REFERENCES


