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

Running head: Mechanisms of ciprofibrate-induced hypergastrinemia

1,3Tom C. Martinsen, 1Ingunn Bakke, 1Duan Chen, 1,3Arne K. Sandvik, 2Kolbjørn Zahlsen, 2Trond Aamo 1,3Helge L. Waldum

1Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, 2Department of Clinical Pharmacology and 3Department of Medicine, St. Olav's Hospital HF, Trondheim University Hospital, Norway
Abstract

Background: 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 PPARα, the mechanism is still not fully elucidated. Methods: Gastric bypass was surgically prepared in male Sprague-Dawley rats. Gastric bypassed and sham-operated rats were either given ciprofibrate (50 mg/kg/day in methocel) or vehicle alone for 7 weeks. PPARα knockout (KO) and wildtype (WT) mice were either given ciprofibrate (500 mg/kg/day in methocel) or vehicle alone for two weeks. The concentration of gastrin in blood was analyzed. Antral G cell density and gastrin mRNA abundance was determined by using immunostaining and Northern blot. Results: 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 3-fold increase in gastrin mRNA in sham-operated rats. In PPARα 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 3-fold increase in gastrin mRNA. Comparing animals dosed with vehicle only, PPARα KO-mice had higher serum gastrin concentration than WT-mice. Conclusions: The main effects of ciprofibrate on G cells are mediated from the antrum lumen and the mechanism is dependent on the PPARα. The results indicate that the PPARα may have a role in the physiological regulation of gastrin release.

Key words: peroxisome proliferator-activated receptor alpha, hypergastrinemia, gastric bypass, PPARα knock-out mice.
**Introduction**

In 1905 Edkins postulated the presence of a humoral gastric acid secretagogue being released from the antral mucosa (19). This was confirmed by Gregory and Tracy in 1961 who identified this secretagogue as gastrin (24). 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 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 man (13, 61). In several animal models hypergastrinemia has induced ECL cell derived gastric tumours (28, 42, 51, 75). Moreover, hypergastrinemic states such as atrophic gastritis and Zollinger-Ellison syndrome are associated with development of gastric tumours (5, 8, 22, 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 content continuously (38, 39). Chemical constituents of the food, especially amino acids, dietary amines (15), calcium (4, 9)
and gastric hypoacidity provide the strongest stimulation of gastrin release (38, 39). H+ ions have 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 non-cholinergic 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). The PPARs have become a topic of intensive research (68) since the discovery of these nuclear steroid receptors by Isseman and Green in 1990 (31). These receptors are found in all organs, but are most abundant in the liver and adipose tissue. Until now, three isoforms of PPARs are demonstrated (α, β, γ) (60) and several PPs are already available for clinical use (20, 64). Stimulators of PPARα have a well-known tumorigenic effect on the rodent liver (53). The exact mechanism is not known, but it seems to be non-genotoxic (48).

The effects of PP on the stomach became an issue of interest due to the findings of ECLomas in the oxyntic mucosa of rats after long-term dosing with PPARα 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 fibrates (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 PPARα agonists was only moderate in contrast to the profound inhibition needed to induce similar ECL cell changes in other models of hypergastrinemia (43). A re-examination 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 weeks of dosing (27) and combination with a proton pump inhibitor revealed a potentiating
hypergastrinemic effect (27). Further, ciprofibrate-induced hypergastrinemia is not reversed by the somatostatin analogue octreotide (3), in contrast to hypergastrinemia caused by inhibition of acid secretion (45). All these findings strongly indicate that the fibrates 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 PPARα since several PPARα agonists, in contrast to a PPARγ agonist (troglitazone), induce hypergastrinemia (1). The demonstration of PPARα immunoractivity 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 PPARα stimulation (49, 67). In ciprofibrate dosed rats we found an increased abundance of ACO mRNA in the antrum, while 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 and PPARα knock-out mice).
Materials and methods

Test substance

Ciprofibrate (2-[4(6-2,2 dichlorocyclopropyl) phenoloxy]-2-methyl propanoic acid) (Sanofi-Synthelabo, Alnwick, UK ) was used as a PPARα 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 (220g body weight) (Møllegaard, Skensved, Denmark) were housed in wire-top cages (5 in each cage) at 20°C and with 40-60 % relative humidity and a 12-h light/dark cycle. The standard rat food pellets (B&K universal, Norway) and tap water were provided ad libitum. Prior to blood sampling the animals were anaesthetized with a subcutaneous injection of 0.02 ml/kg of a solution containing 2.5 mg/ml fluanison, 0.05 mg/ml fentanyl (Tamson Animal Health, Buckinghamshire, UK) and 1.25 mg/ml midazolam (Alpharma AS. 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 oesophagus to the first segment of duodenum end-to-side, leaving the stomach connected to the duodenum, but not to the oesophagus. The sham operation included laparotomy through a mid-line abdominal incision followed by gentle manipulation of the viscera. The animals were sacrificed by puncture of great vessels close to the heart under anaesthesia.
**Mice**

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

**Study design**

**Rats**

Two groups of gastric bypassed (n=8 in each group) and two groups of sham-operated rats (n=8 in each group) were either given ciprofibrate 50 mg/kg in methocel, a dose previously shown to induce hypergastrinemia in rats (3, 43) or 0.5 ml of vehicle alone by daily gastric intubation for 7 weeks. Unfortunately, among the gastric bypassed animals, one individual in the ciprofibrate group and three in the methocel died during the first week of the study. Consequently, there were 7 animals in the ciprofibrate group and 5 animals in the methocel group at 4 weeks. Between 4 and 7 weeks some more gastric bypassed animals died and at termination of the study (after 7 weeks of dosing) there were 4 animals in each group. Blood was collected from the femoral vein at the start of the study, after 4 weeks and after 7 weeks. Plasma was frozen at -20°C for later determination of gastrin concentration by radioimmunoassay (RIA) (33) and ciprofibrate concentration by high pressure liquid
chromatography (HPLC). At sacrifice, the stomachs were immediately removed and opened along the greater curvature and rinsed in saline. Samples were collected from the antral mucosa at a standardised location and fixed in 4% buffered formaldehyde or homogenised for RNA extraction. The homogenates were frozen at -80°C for later processing.

**Mice**

Two groups of PPARα KO-mice and WT-mice were either given ciprofibrate 500 mg/kg 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 two weeks. Ciprofibrate induced hypergastrinemia has previously found to be only transient in mice (18), however the dose used (20 mg/kg/day) 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 to rats. By using 500 mg/kg/day, mice became hypergastrinemic after only two weeks. At sacrifice, 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 and opened along the greater curvature and rinsed in saline. Samples were collected from the antral mucosa at a standard location and fixed in 4% buffered formaldehyde or homogenised 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 mucosa 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, Dako Glostrup, Denmark) (dilution1:700) for 2 hours at room temperature followed by the
EnVision-HRP technique (K5007, Dako). The immunoreactivity was detected using the AEC peroxidase kit (SK4200, Vector Laboratories, Inc. Burlingame, CA). As negative control, the primary antiserum was substituted with diluted non-immune 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 (IR) cells per millimetre 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 (250µm) were counted.

**Northern blot analysis**

Biopsies for RNA analysis were homogenized (Ultra-Turrax homogenizer, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) in a denaturing buffer (1ml/100 mg tissue) containing guanidinium isothiocyanate (4 M), sodium acetate (25 mM, pH 6.0) and β-mercaptoethanol (0.84% v/v). 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 18S ribosomal RNA were made as previously described (54). Plasmids were linearized and antisense RNA probes simultaneously transcribed and labeled with $^{32}$P ($^{32}$P-CTP, Amersham International, Bucks, UK) according to standard protocols. The probes were purified on NICK columns (Pharmacia Biotech AB, Uppsala, Sweden).

Northern blots were prepared by electrophoresing total RNA (20 µg/well) in 1% agarose-formaldehyde gels, electroblotting onto nylon membranes (Roche, Mannheim, Germany) and crosslinking by UV irradiation. The membranes were prehybridized for 4 hours at 65°C in 5×
standard saline phosphate EDTA (SSPE) buffer (1× SSPE is 0.18 M NaCl, 10 nM NaH₂PO₄ and 1 mM EDTA (pH 7.4)), with 50% formamide, 5× Denhart’s solution (0.1% bovine serum albumine, polyvinylpyrroldone and Ficoll 400), 0.5% sodium dodecyl sulphate (SDS) and sonicated salmon sperm DNA. The ³²P-labeled cRNA probe (2 × 10⁶ cpm/ml) was added for overnight hybridization at 65°C. The blots were washed twice in 2× SSPE with 0.1% SDS for 20 min at room temperature and once in 0.1× SSPE with 0.1% SDS at 65°C, exposed to a storage phosphor screen for 24 hours, scanned on a PhosphorImager (BAS-1800II, FujiFilm, Kanawaga, Japan), and quantified using the ImageGauge software (Version 3.4x, Science Lab 99, Fuji Photo Film, Kanagawa, Japan). Stripping between hybridizations was done with boiling 0.1% SDS. Sizes of the different mRNAs were estimated with reference to 18S and 28S ribosomal RNA, 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 with the mouse sequences and the size of the different mRNAs as measured on Northern blot were as expected for the mouse.

In the analysis of mRNA abundance, sham-operated rats and WT-mice dosed with vehicle alone were set as control.

Statistics

One-way analysis of variance (ANOVA) with Bonferroni’s post-test was used to statistically evaluate three or more groups, while unpaired t-test was used to compare two groups. For all evaluations P>0.05 was considered non-significant. All values are given as mean ± standard error of the mean (SEM).
Results

Plasma/serum concentrations of gastrin

*Rats (fig. 1A)*

After 7 weeks ciprofibrate did not raise plasma gastrin concentration significantly in gastric bypassed rats compared to 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 2-fold 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). Comparing animals dosed with vehicle only, the gastric bypass operation induced more than a 50% decrease in the plasma gastrin concentration (p<0.05). Neither after 4 weeks did ciprofibrate raise plasma gastrin concentration in gastric bypassed rats compared to 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 similar to the concentrations found after 7 weeks, revealing exactly the same differences when comparing different groups of rats (data not shown).

*Mice (fig. 2A)*

In PPARα KO-mice, ciprofibrate induced a decrease in serum gastrin concentration compared to vehicle (17.5 ± 1.9 vs 27.4 ± 3.5 pmol/L, p<0.05). In contrast, in WT-mice ciprofibrate induced a 2-fold increase in serum gastrin concentration compared to 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 PPARα KO-mice dosed with ciprofibrate (p<0.001). Comparing animals dosed with vehicle only, PPARα KO-mice had higher serum gastrin concentration than WT-mice (p<0.05).
Plasma/serum concentrations of ciprofibrate

**Rats**

After 7 weeks the concentration in bypassed rats were higher than in sham-operated rats (2276 ± 240 vs 1443 ± 96 µmol/L, p<0.01). After 4 weeks there was no difference in plasma concentration of ciprofibrate in bypassed rats and sham-operated rats dosing (1976 ± 179 vs 1850 ± 112 µmol/L).

**Mice**

There was no difference in serum concentration of ciprofibrate in PPARα KO-mice and WT-mice (1510 ± 73 vs 1763 ± 152 µmol/L).

Antral G cell density

**Rats (fig. 1B)**

In gastric bypassed rats, ciprofibrate did not raise antral G cell density compared to vehicle (7.7 ± 0.6 vs 8.3 ± 0.5 number of G cells/mm mucosa length). In contrast, in sham-operated rats, ciprofibrate induced an approximately 50% increase in antral G cell density compared to vehicle (14.8 ± 0.6 vs 9.5 ± 1.0 number of G cells/mm mucosa length, p<0.001). The antral G cell density in ciprofibrate dosed sham-operated rats was 2-fold the antral G cell density found in ciprofibrate dosed gastric bypassed animals (p<0.001). Comparing animals dosed with vehicle only, there was no difference in antral G density.

**Mice (fig. 2B)**

In PPARα KO-mice, ciprofibrate did not increase antral G cell density compared to vehicle (5.8 ± 0.3 vs 5.8 ± 0.3 number of G cells/mm mucosa length). In contrast, in WT-mice, ciprofibrate induced nearly a 2-fold increase in antral G cell density compared to vehicle (11.5 ± 0.6 vs 6.5 ± 0.4 number of G cells/mm mucosa length, p<0.001).
WT-mice dosed with ciprofibrate had nearly two times the antral G cell density found in PPARα KO-mice dosed with ciprofibrate (p<0.001). There was no difference in antral G cell density between PPARα KO-mice and WT-mice dosed with vehicle.

**Antral gastrin and somatostatin mRNA expression**

**Rats (fig. 3)**

In gastric bypassed rats ciprofibrate induced a significant increase in the antral abundance of gastrin mRNA (fig. 3A) compared to vehicle (95 ± 23 vs 36 ± 8 % of control (sham-operated rats given methocel), p<0.05). In sham-operated rats ciprofibrate induced nearly a 3-fold increase compared to 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). Comparing animals dosed with vehicle only, the bypass operation induced more than 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 towards increased abundance in sham-operated rats receiving ciprofibrate (142 ± 18 % of control) compared to vehicle dosing (p=0.057) and compared to 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 (fig. 4)**

In PPARα KO-mice, ciprofibrate did not increase the antral abundance of gastrin mRNA (fig. 4A) compared to vehicle (124 ± 19 vs 110 ± 12 % of control). In contrast, in WT-mice ciprofibrate induced more than a 3-fold increase in the antral abundance of gastrin mRNA compared to 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 PPARα
KO-mice dosed with ciprofibrate (p<0.01). There was no difference in antral abundance of gastrin mRNA between PPARα 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 towards increased abundance after ciprofibrate dosing (151 ± 26 % of control, p=0.074). Comparing KO-mice and WT-mice dosed with vehicle, the level of somatostatin mRNA seems 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 PPARα KO-mice, ciprofibrate had no effect on liver weights compared to vehicle (0.90 ± 0.03 vs 0.70 ± 0.04 g). In contrast, in WT-mice, ciprofibrate induced more than a four-fold 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 PPARα 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). Further, G cell hyperfunction is not reversed by the normally suppressive somatostatin analogue 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 two weeks 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.

The 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 weeks. At 7 weeks the number of animals was reduced due to 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 can not be excluded. 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 number of immunoreactive G cells per mm 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 cells 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 cells 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, 21). 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). Since 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 the PPARα (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 to 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 weeks 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. Of an unexplained cause, the plasma concentration of ciprofibrate at sacrifice was lower in the sham animals compared to the gastric bypassed animals. This may be explained by gastric destruction. However, in spite of a higher plasma concentration of ciprofibrate in the gastric bypassed animals at sacrifice, 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 sacrifice were equal in both PPARα KO- and WT-animals, reflecting that the substance is absorbed equally in the two groups.

**Hypergastrinemia induced by PPARα agonists – not only in rats**

The hypergastrinemia induced by fibrates has so far only been consistently observed in rats. Indeed, Eason et al. reported a transient hypergastrinemia in mice, while there was no effect on serum gastrin in marmoset (18). Accordingly, the histological changes in gastric mucosa have only been shown in rats. However, in the previous studies the same dose per kg 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, emphasising interspecies variation in pharmacokinetics. Consequently, the G cell stimulating effects of PPARα agonists is not restricted to the rat and may possible also be found in humans. The recommended daily dose of ciprofibrate in man to
treat hyperlipidemia is usually 100 mg which is approximately 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 can not be excluded that a similar G cell stimulating effect could be found in patients using clinical doses of ciprofibrate.

*The PPARα in regulation of gastrin release*

Since ciprofibrate is regarded as one of the most specific PPARα agonist, its G cell stimulatory effect has been suspected to be PPARα dependent. In recent studies this suggestion has been substantiated by several findings. First, another specific non-fibrate PPARα agonist WY 14643 was also found to induce hypergastrinemia (1), whereas the PPARγ 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 PPARα stimulation (49, 67). Third, PPARα immunoreactivity has been demonstrated in G cells (1), supporting a role of this receptor in G cell stimulation.

The impact of the PPARα in ciprofibrate-induced G cell stimulation is clearly demonstrated by the use of PPARα 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 PPARα KO-mice. However, ciprofibrate induced a significant decrease in serum gastrin level in these mice. This indicates that in absence of the PPARα, 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 PPARα 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 PPARα KO animals. This finding is probably not due to methocel since unpublished results from our laboratory confirm that serum gastrin level in PPARα KO-mice not dosed with methocel is higher than in WT-mice. Knowing the hypergastrinemic effect of ciprofibrate acting on PPARα, this finding was unexpected. There were no differences in antral density of G cells or antral gastrin mRNA abundance between methocel dosed PPARα KO- and WT-mice. Thus, according to these results, the lack of PPARα seems to influence gastrin release without affecting G cell density or gastrin gene expression. An alternative explanation may be that PPARα influences enzymes required for progastrin processing. This remains to be settled.

As a control of the KO-model, the liver weights were measured at sacrifice showing more than a three-fold 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 liver induced by PPARα 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 PPARα which leads to a heterodimerisation with an activated 9-cis retinoic acid receptor (RXR) (41). Subsequently, the ligand receptor complex acts as a transcription factor by binding to DNA (40). The PPARα 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 the PPARα 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, being 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 the PPARα. Like the other PPARs, the ligand binding domain of the α-isoform 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 towards 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 PPARα KO and WT animals, it seems reasonable to assume that the PPARα 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 man. The only human study on this subject showed an increase in 24h intragastric acidity after oral administration of clofibrate (23). There was no effect on serum gastrin during the study. From this 24h 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 non-amidated gastrin
precursors are increased in patients with such disease (11). Recently, several PPARα agonists were shown to stimulate progastrin production in a human colorectal cell line (LIM 1899) (35).

In conclusion (fig. 5), by the use of gastric bypassed rats, the main stimulatory effects of ciprofibrate on G cells are found to be mediated locally in the antrum. By the use of PPARα KO-mice the main stimulatory effects of ciprofibrate on G cells are found to be mediated by PPARα activation.
Figure legends

Figure 1.
Mean plasma gastrin concentration (pmol/l) ± SEM (A) and density (number of cells / mm mucosa length) ± SEM of cells immunoreactive for gastrin in antrum (B) in sham operated and gastric bypassed rats given either ciprofibrate (50 mg/kg) or methocel (vehicle) by daily intubations for 7 weeks. * p<0.05, ** p<0.01, *** p<0.001.

Figure 2.
Mean serum gastrin concentrations (pmol/l) ± SEM (A) and density (number of cells / mm mucosa length) ± SEM of cells immunoreactive for gastrin in antrum (B) in wildtype (WT) and PPARα knockout (KO) mice given either ciprofibrate (500 mg/kg) or methocel (vehicle) by daily intubations for 2 weeks. * p<0.05, *** p<0.001.

Figure 3.
Mean antral gastrin (A) and somatostatin (B) mRNA abundance ± SEM in sham operated and gastric bypassed rats given either ciprofibrate (50 mg/kg) or methocel (vehicle) by daily intubations for 7 weeks; expressed as per cent of values in control animals (sham operated rats given methocel). * p<0.05.

Figure 4.
Mean antral gastrin (A) and somatostatin (B) mRNA abundance ± SEM in wildtype (WT) and PPARα knockout (KO) mice given either ciprofibrate (500 mg/kg) or methocel (vehicle) by daily intubations for 2 weeks; expressed as per cent of values in control animals (WT-mice given methocel). ** p<0.01.

Figure 5.
The mechanism of ciprofibrate-induced antral G cell stimulation; a local effect on an open cell by activating nuclear PPARα; stimulation (+) and inhibition (-) of gastrin release.
Acknowledgements:

We thank Bjørn Munkvold, Britt Schulze and Anne Kristensen for their technical assistance.
References


Figure 1A

**Plasma gastrin [pmol/L]**

- **Methocel**
- **Ciprofibrate**

**Sham**
- Methocel
- Ciprofibrate

**Bypass**
- Methocel
- Ciprofibrate


Figure 1B

**Gastrin IR in antrum**

- **Methocel**
- **Ciprofibrate**

**Sham**
- Methocel
- Ciprofibrate

**Bypass**
- Methocel
- Ciprofibrate
Figure 2A

Serum gastrin [pmol/L]

WT

KO

Methocel

Ciprofibrate

Figure 2B

Gastrin IR in antrum [number of cells / mm mucosa length]

WT

KO

Methocel

Ciprofibrate
Figure 3A

Antral gastrin mRNA abundance (% of control)

- Methocel
- Ciprofibrate

Sham | Bypass
---|---

* * *

p = 0.089

Figure 3B

Antral somatostatin mRNA abundance (% of control)

- Methocel
- Ciprofibrate

Sham | Bypass
---|---

* * *

p = 0.057 p = 0.089
Antral gastrin mRNA abundance (% of control)

- **Methocel**
- **Ciprofibrate**

WT KO

** p = 0.074

Antral somatostatin mRNA abundance (% of control)

- **Methocel**
- **Ciprofibrate**

WT KO

p = 0.087

p = 0.074
Figure 5

G cell

Lumen of antral gland

PPARα

Transcription

Gastrin

Food

Ciprofibrate

H’