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Ghrelin and Motilin Are Cosecreted from a Prominent Endocrine Cell Population in the Small Intestine

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Context: Ghrelin is a novel hormone produced mainly in the gastric body. Hitherto, mapping studies of ghrelin cells covering the entire gastrointestinal (GI) tract in humans have been lacking. Furthermore, the phenotype of extragastric ghrelin cells is not known.

Objective: The objective of the study was to perform a detailed mapping with specimens from all parts of the GI tract, and colocalization studies to phenotype ghrelin cells along the tract. In addition, mapping of ghrelin cells was performed in porcine GI tract, and the plasma profiles of ghrelin and motilin in blood from the porcine intestine were measured.

Design: Biopsies from patients were obtained during gastroscopy or surgery. Ghrelin cell density and phenotyping was assessed with immunocytochemistry, *in situ* hybridization, and immunogold electron microscopy. Plasma ghrelin and motilin levels were measured in pigs, fitted with cannulas in the mesenteric vein.

Results: The upper small intestine is unexpectedly rich in ghrelin cells, and these cells contribute to circulating ghrelin. Ghrelin and motilin are coproduced in the same cells in the duodenum and jejunum of both species, and ghrelin and motilin are stored in all secretory granules of such cells in humans, indicating cosecretion. The plasma profiles of ghrelin and motilin in pig were parallel, and a correlation between ghrelin and motilin ($r^2 = 0.22$; $P < 0.001$) was evident in intestinal blood.

Conclusions: The upper small intestine is an important source of ghrelin. The likely cosecretion of intestinal ghrelin and motilin suggests concerted actions of the two hormones. These data may have implications for understanding gut motility and clinical implications for dysmotility and bariatric surgery. (***J Clin Endocrinol Metab* 92: 3573–3581, 2007**)

AN ARRAY OF STUDIES have demonstrated that the novel hormone ghrelin (1) affects several bodily functions, including appetite, food intake, fat utilization, and body weight (2, 3). Importantly, circulating ghrelin is increased by fasting and decreased postprandially (4–6). Recently the fasting-induced rise in gastric ghrelin expression and plasma ghrelin were suggested to be mediated via cholecystokinin (CCK) in mice (7). Ghrelin also affects gastrointestinal (GI) functions. Thus, stimulatory effects of ghrelin on gastric emptying (8–11), intestinal motility (12–14), and acid secretion (8, 10, 15) have been observed in rodents. It should, however, be mentioned that ghrelin knockout mice display normal gastric emptying (16). Interestingly, ghrelin induces GI motility also in man (3, 17–20). In the oxyntic mucosa ghrelin is produced by A-like cells in the rat and mouse, and P/D1 cells in humans (6, 21, 22). In mouse and rat, ghrelin cells are most abundant in the oxyntic mucosa, less frequent in the gastric antrum, and still fewer in the small intestine (6, 21, 23). In humans 35–45% of plasma ghrelin remains after total gastrectomy (24, 25). Thus, although the stomach is the major source of ghrelin, other sources clearly exist. One such potential source is the small intestine; another is the pancreas. In the pancreatic islets, ghrelin is found in a novel cell type, which in humans resembles P/D1 cells (26, 27); islet ghrelin cells are also evident in mice (28, 29) and rats (30) during development.

There are reports suggesting that ghrelin cells in the human small intestine are rare or infrequent (21, 22). However, no detailed mapping of ghrelin cells, covering the entire human GI tract, has been performed. We therefore studied ghrelin immunoreactive (IR) cells and their distribution in relation to other endocrine cells in all parts of the human GI tract. To phenotype the ghrelin cells, the relationship between ghrelin cells and motilin cells was given special attention. In preliminary studies we observed colocalization of ghrelin and motilin in duodenal biopsies. We therefore extended the phenotyping to porcine small intestine to find a suitable animal model for secretion studies. The plasma profiles of ghrelin and motilin in the porcine mesenteric vein after feeding were examined.

Patients and Methods

Human tissues and tissue processing

Specimens were taken from the stomach and proximal duodenum during gastroscopy in 16 patients, terminal ileum and colon during colonoscopy in 16 patients, and the jejunum at bariatric surgery in nine patients (29 women and eight men; median age 46 yr; age range 18–82 yr). Some patients had more than one procedure performed. Specimens were taken from reference points, *e.g.* the major curvature in the middle of corpus, the prepyloric area of the antrum, proximal duodenum (10 cm from duodenal bulb), proximal jejunum, and terminal ileum and from the middle part of the caecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. The studies were approved

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Abbreviations: CCK, Cholecystokinin; GI, gastrointestinal; GIP, glucose-dependent insulinotropic polypeptide; IR, immunoreactive; MMC, migrating motor complex.

by the human ethics committee in Lund. The specimens were collected from patients examined for different clinical reasons as a diagnostic procedure, but only those displaying a macroscopically and microscopically normal mucosa in routine histological stainings were selected for the study (Table 1). Specimens were immediately fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.2) at 4 C for 24–48 h, rinsed in Tyrode's solution containing 10% sucrose, and frozen at −80 C. Sections (10 μm) were cut in a cryostat and mounted on slides. Fetal pancreatic specimens were used as previously described (26).

Porcine tissues and tissue processing

Male pigs (Swedish Landrace × Yorkshire × Hampshire; aged 8–10 wk; body weight 13.5 ± 1.5 kg, n = 6) were maintained on a 12-h day, 12-h night rhythm and housed individually in 1.0 m × 1.5 m pens, equipped with a dry feeding trough and a drinking nipple. Pigs had free access to tap water and were fed two meals (standard pig food; 2% of body weight/meal) a day. The pigs were killed using pentobarbital 60 mg/kg. Specimens from corpus and antrum of the stomach, duodenum, jejunum, and ileum of the small intestine and colon of the large intestine were taken and processed as described above. The experiments were approved by the regional animal ethics committee.

Immunocytochemistry

Antibodies were diluted in PBS (pH 7.2) containing 0.25% BSA and 0.25% Triton X-100. Sections were incubated with primary antibodies (Table 2) overnight at 4 C, followed by rinsing in PBS with Triton X-100 for 2 × 10 min. Thereafter secondary antibodies with specificity for rabbit, guinea pig, mouse, or goat IgG and coupled to either fluorescein isothiocyanate or Texas-Red (Jackson, West Grove, PA) were applied on the sections. Incubation was for 1 h at room temperature. Sections were again rinsed and then mounted in PBS-glycerol, 1:1. In addition, ghrelin antibodies, directly conjugated with 5- (and 6-) carboxyfluorescein, were used (Table 2). The specificity of immunostaining was tested using primary antisera preabsorbed with excess amount of homologous antigen (100 μg of peptide per milliliter antiserum in working dilution) or by omission of primary antibodies.

Cell density quantification

The density of IR cells was quantified in a fluorescence microscope (visual field area = 0.63 mm^2) as previously described (31). Briefly, the number of immunoreactive cells was counted in transversely sectioned mucosa, with the entire depth of the mucosa visible, in two to 12 visual fields in each of at least three sections from each specimen. The densities were expressed as cell number per square millimeter mucosa.

In situ hybridization

A probe (26), complementary to the sequence 153–182 of human preproghrelin mRNA (accession no. NM 016362) (1) was 3′-end tailed with [^{35}S]dATP (PerkinElmer, Stockholm, Sweden), and *in situ* hybridization was performed as previously described (32). Controls included use of sense probe or hybridization in the presence of 100-fold excess of unlabeled probe.

TABLE 1. Number of patients studied for each segment of the GI tract

Localization of specimens	No. of patients
Stomach	
Corpus	8
Antrum	7
Duodenum	7
Jejunum	9
Ileum	5
Colon	
Caecum	5
Ascending	5
Transverse	4
Descending	5
Sigmoid	9
Rectum	8

Electron microscopy and immunogold

Specimens were fixed and embedded in Lowicryl HM20 (TAAB, Reading, UK), as previously described (33). Consecutive ultrathin sections were placed on several gold grids. Sections were blocked with PBS (pH 7.2) containing 0.5% BSA, incubated overnight at 4 C with primary antisera (ghrelin, code 486–1, dilution 1:200; motilin, code R1105, dilution 1:200; Table 2), diluted in PBS containing 0.25% BSA and 0.25% Triton X-100, washed in PBS, and thereafter incubated for 1 h at room temperature with protein A-gold solution (10 nm diameter, dilution 1:20; Amersham Pharmacia Biotech, Uppsala, Sweden) and again washed in PBS (33). Grids were contrasted with 4% uranyl acetate and 0.5% lead citrate before examination in a CM10 transmission electron microscope (Philips, Best, The Netherlands). Diameter of secretory granules (dense core) was measured as previously described (27).

Secretion study

Surgery. The pigs were anesthetized using a 0.5–1.5% air mixture of Fluothane (AstraZeneca, Göteborg, Sweden) and carrier O_2 at approximately 0.5 liters/min. They were intubated, placed on a surgical table, and disinfected with iodine solution and 70% ethanol. A 14- to 18-cm-long incision was made from the sternum along the linea alba. Silicon catheters (Dow Corning, Midland, MI; outside diameter 1.64 mm, inner diameter 0.75 mm) were implanted in the mesenteric vein. To selectively sample blood from the intestine, and avoiding blood from the stomach, the catheters were placed in the superior mesenteric vein distally to the junction with the gastroepiploic vein. One catheter ran via a liver vein with its tip placed in the superior mesenteric vein, 2–3 cm distally to the junction with the gastroepiploic vein. The second catheter was placed directly into superior mesenteric vein against the bloodstream 3–4 cm distal to the junction with the gastroepiploic vein. In addition, a catheter was placed in the right jugular vein to allow sampling of systemic blood. After surgery, the pigs were treated with ampicillin (Doktacillin; Astra Läkemedel, Södertälje, Sweden) 500 mg daily for 3 d. Within 2–3 d, the pigs recovered from surgical stress and displayed normal food (4% of body weight per day) and water intake as well as normal (2.5) ratio between daily food intake and daily body weight gain.

Experimental design

Experiments were performed 5 d after surgery. The pigs were fasted for 24 h before the experiments. Fasting blood samples were taken. The pigs were then given a standard meal and blood samples were collected 10, 20, 30, 40, 50, 60, 90, and 240 min subsequent to the meal. Blood samples were collected in ice-cold tubes containing aprotinin (Trasylol, 10,000 KU/ml; Bayer, Leverkusen, Germany) and EDTA (0.4 g/ml), centrifuged (2000 × *g* × 4 C, 20 min), and stored at −80 C until analysis.

Autopsy

All pigs were killed the day after the experiment and examined to verify that the tips of all catheters were located at the right place.

Plasma analysis

The concentrations of total ghrelin (acylated and nonacylated) and motilin were determined using RIA (Phoenix, Belmont, CA). Intraassay variation was less than 1%.

Statistical analysis

Results are shown as mean ± SEM. Plasma data were analyzed using Student's paired *t* test. Linear regression analysis was performed with Spearman's test. Differences with a value of $P < 0.05$ were considered significant.

TABLE 2. Details of the different antisera used

Hormone	Code	Dilution	Source
Ghrelin	486-1	1:2560	Phoenix (Belmont, CA)
Ghrelin	726-2	1:2560	Phoenix
Ghrelin	H-031-31	1:3200	Phoenix
Ghrelin	FG-G-031-31	1:1000	Phoenix
Ghrelin	SC10368	1:1000	Santa Cruz Biotechnology Inc. (Santa Cruz, CA)
Motilin	8422	1:800	EuroDiagnostica (Malmö, Sweden)
Motilin	GP1103	1:6400	Kind gift from Prof. N. Yanaihara (Shizuoka, Japan)
Motilin	R1105	1:800	Kind gift from Prof. N. Yanaihara
Gastrin	7835	1:1280	EuroDiagnostica
Glucagon	7811	1:5120	EuroDiagnostica
Somatostatin	V 1169	1:400	Biomeda (Foster City, CA)
Chromogranin A	LK2H10	1:300	Boehringer (Mannheim, Germany)
GIP	11/19/77	1:640	Kind gift from Prof. T. O'Dorisio (Columbus, OH)
Secretin	7875	1:640	EuroDiagnostica
Serotonin	20080	1:1600	Dia Sorin Ink (Stillwater, MN)
Neurotensin	HC8	1:640	Kind gift from Prof. R. Carraway (Boston, MA)

Results

Human studies

Mapping and quantification of ghrelin cells in the human GI tract

Immunocytochemistry: stomach. In the corpus/fundus region, numerous strongly ghrelin IR cells were seen from base to neck of the oxyntic glands, as expected (Fig. 1A and Table 3). In the antrum, ghrelin cells were very few and displayed only weak to moderate immunoreactivity (Fig. 1B and Table 3); gastrin cells were numerous as expected (Table 3).

Immunocytochemistry: small and large intestine. A substantial number of ghrelin IR cells was also found in the duodenal mucosa (Fig. 1C). Quantification revealed that the density of ghrelin IR cells was similar to the density of motilin IR cells (Table 3). In the jejunal mucosa the densities of both ghrelin IR cells and motilin IR cells were comparable to those in the duodenum (Fig. 1D and Table 3). In ileum ghrelin IR cells were very few, and in the different regions of colon and rectum examined, ghrelin IR cells were undetectable (Table 3).

In situ hybridization. To confirm our immunocytochemical data, *in situ* hybridization for ghrelin mRNA was performed. Labeling for ghrelin mRNA was abundant in the oxyntic mucosa of the stomach, thus confirming that the probe was efficient. Only very few cells with labeling for ghrelin mRNA were detected in the antral mucosa, whereas such cells were frequently seen in the duodenal and jejunal mucosa. Results are summarized in Fig. 2 and Table 3. Control sections displayed only weak randomly scattered labeling (data not shown).

Colocalization study. Next, we aimed to examine the cellular identity of the intestinal ghrelin IR cells. This was addressed

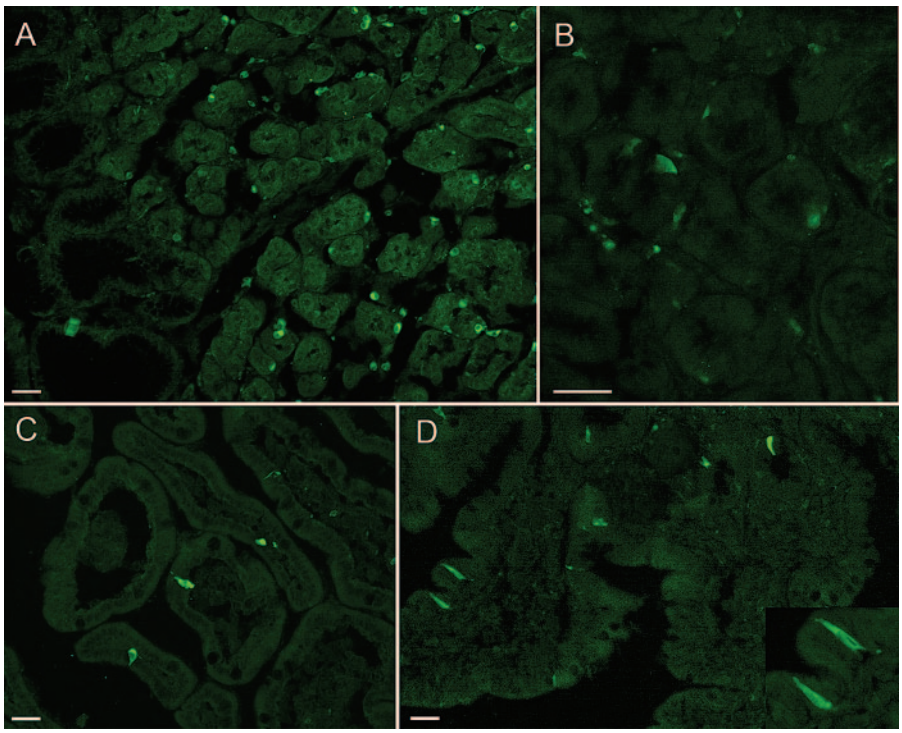

FIG. 1. Immunofluorescence micrographs of human GI tract, immunostained for ghrelin (726–2). Ghrelin cells are numerous in the oxyntic mucosa of the stomach (A) but few in the antrum (B). A substantial number of ghrelin IR cells are seen in duodenum (C) and jejunum (D). *Scale bars*, 50 μm.

TABLE 3. Density of ghrelin cells in the human GI tract and their relation to other endocrine cells as chromogranin A-positive cells in corpus, gastrin cells in antrum and motilin cells in duodenum and jejunum

Localization	Ghrelin		Motilin	Chromogranin A	Gastrin
	ICC	ISH	ICC	ICC	ICC
Stomach					
Corpus	78 ± 13	45 ± 7	0	160 ± 27	0
Antrum	<1	<1	0		>300
Duodenum	14 ± 1	4 ± 1	16 ± 3		
Jejunum	14 ± 1	5 ± 1	16 ± 2		
Ileum	<1	<1	<1		
Colon					
Caecum	0	0			
Ascending	0	0			
Transverse	0	0			
Descending	0	0			
Sigmoid	0	0			
Rectum	0	0			

Data are expressed as cells/mm^2 mucosa. All figures are from mucosa with normal routine histology. Ghrelin cells were detected with both immunocytochemistry (ICC) and *in situ* hybridization (ISH).

by studying colocalization of ghrelin and other gut hormones. Double immunostainings for ghrelin/glucagon, ghrelin/somatostatin, ghrelin/CCK, ghrelin/glucose-dependent insulinotropic polypeptide (GIP), ghrelin/secretin, ghrelin/serotonin, and ghrelin/neurotensin revealed that ghrelin IR cells were separate from L cells, D cells, I (CCK) cells, K (GIP) cells, S (secretin) cells, EC cells, and neurotensin cells. Interestingly, however, double staining for ghrelin and motilin revealed that almost all (96 ± 2%) ghrelin IR cells in the duodenum were also motilin IR (Fig. 3, A–C). Only 3 ± 1% of the motilin IR cells were devoid of ghrelin, and 1 ± 1% of the ghrelin IR cells lacked motilin IR. Similarly, in the jejunum 89 ± 2% of the ghrelin IR cells were also motilin IR (Fig. 3, D–F); 2 ± 0.2% of the ghrelin IR cells were devoid of motilin and 9 ± 1.5% of the motilin IR cells lacked ghrelin IR. The colocalization of ghrelin and motilin was confirmed with three different motilin antisera and several different ghrelin antisera. To test whether the colocalization was specific for the intestine, we double stained for ghrelin and motilin in the stomach and fetal pancreas. No motilin IR could be detected in gastric (Fig. 3, G–I) or islet ghrelin cells (Fig. 3, J–L).

Electron microscopy and immunogold labeling for ghrelin and motilin in duodenum. To examine further the cellular identity of the ghrelin and motilin coexpressing cells, electron microscopy with immunogold labeling for ghrelin and motilin was used. Figure 4 illustrates cells in the duodenal mucosa labeled for both motilin (Fig. 4, A, C, E, G, I, and K) and ghrelin (Fig. 4, B, D, F, H, J, and L), in consecutive ultrathin sections. Both antisera labeled virtually all the secretory granules (ghrelin: 95 ± 1%; motilin 93 ± 4%), implying coexistence of ghrelin and motilin also at the level of the secretory granules. Morphometrical analysis revealed that the dense cores of the secretory granules of the ghrelin IR/motilin IR cells had a mean diameter of 143 ± 8 nm.

Porcine studies

Mapping of ghrelin cells in the porcine GI tract. Immunostaining for ghrelin confirmed that the corpus region of the stomach displayed numerous (200 ± 4 cells/mm^2) ghrelin IR cells as previously reported (34). The antrum harbored fewer cells (10 ± 1 cells/mm^2). Both duodenum and jejunum harbored 13 ± 2 ghrelin IR cells/mm^2. Double immunostainings for ghrelin and glucagon, somatostatin, CCK, GIP, secretin, serotonin, or neurotensin revealed that ghrelin was not colocalized with any of these gut hormones. Double immunostaining for ghrelin and motilin revealed that 83 ± 3% of the ghrelin IR cells in the duodenum were identical with motilin cells (Fig. 5, A–C); in the jejunum the corresponding figure was 61 ± 4% (Fig. 5, D–F). Ghrelin IR cells were rare (<1 cell/mm^2) in the ileum and lacking in the colon. *In situ*

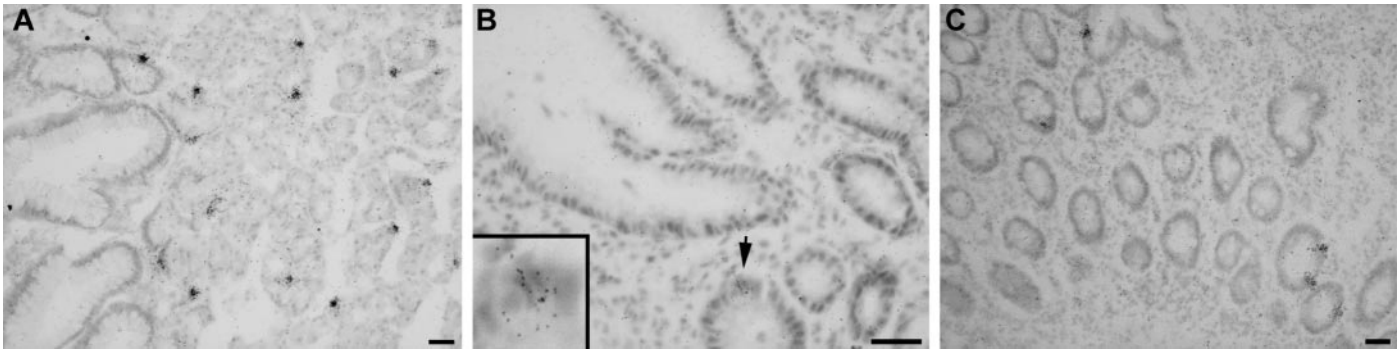

FIG. 2. *In situ* hybridization for ghrelin mRNA of human stomach and intestinal mucosa. Numerous cells with labeling for ghrelin mRNA are seen in the oxyntic mucosa of the stomach (A). Few cells with ghrelin mRNA expression are seen in the antrum (B); cell indicated by *arrow* is shown in higher magnification to visualize labeling. Cells with labeling for ghrelin mRNA are readily seen in the duodenum (C). *Scale bars*, 50 μm.

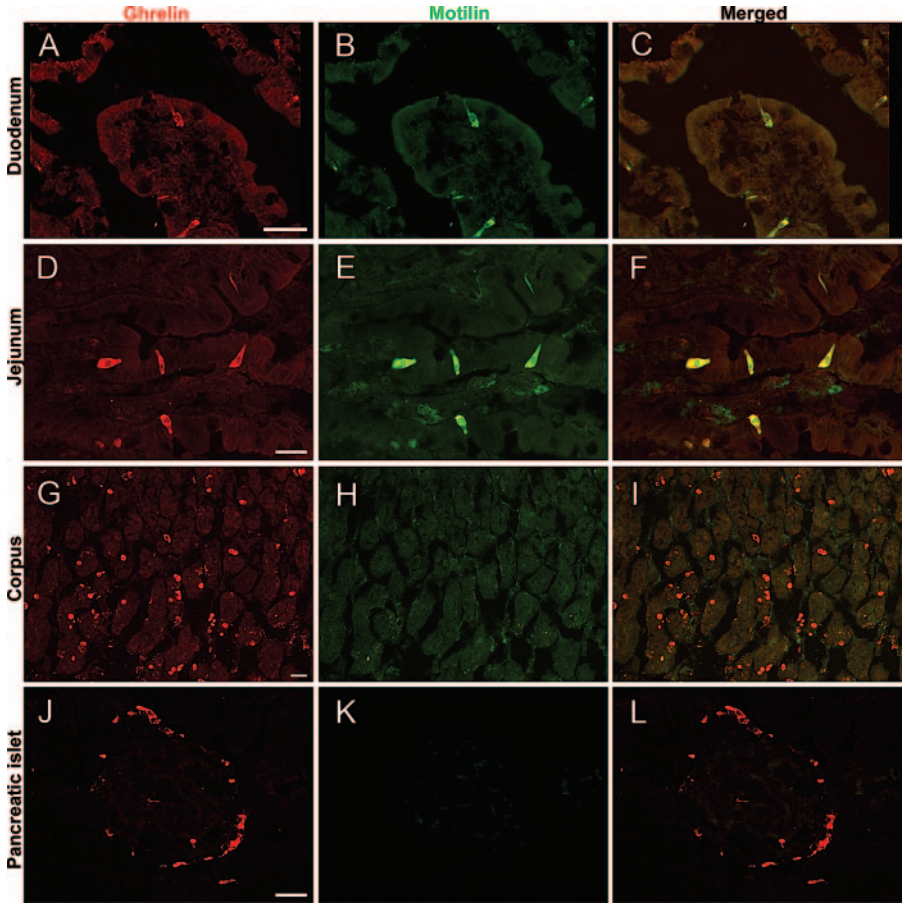

FIG. 3. Immunofluorescence micrographs of human GI tract and fetal pancreas, double immunostained for ghrelin (726–2) (*red*, *left*); motilin (GP1103) (*green*, *middle*); merged (*right*). Ghrelin and motilin are colocalized in the same cells in the duodenum (A–C) and jejunum (D–F). No motilin immunoreactivity is seen in the ghrelin cells in the oxyntic mucosa of the stomach (G–I) or in the pancreatic islets (J–L). *Scale bars*, 50 μm.

hybridization for ghrelin mRNA confirmed the immunocytochemical data (not shown).

Secretion study. Because ghrelin and motilin were costored in the same granules, we hypothesized that the two peptides are cosecreted. We found the pig to be a suitable animal model because the pattern of colocalization of ghrelin and motilin in the porcine intestine was very similar to that of the human intestine. We therefore aimed at investigating ghrelin and motilin plasma profiles in blood emanating from the intestine. Because the oxyntic mucosa of the stomach is the major source of circulating ghrelin, we needed to sample blood from a position in the mesenteric vein located distally to the entrance of venous blood from the stomach. To this end, pigs were catheterized with cannulas into the mesenteric vein 2–4 cm distal to the junction with the gastroepiploic vein. In addition, catheters were placed in one of the jugular veins for sampling of systemic blood. The pigs were fasted for 24 h, and blood samples were taken. The fasting ghrelin concentration was more than 3-fold higher in mesenteric blood than systemic blood (mesenteric: 435 $\pm$ 62 pg/ml; systemic: 139 $\pm$ 40 pg/ml; $P < 0.003$; Fig. 6A). There was no difference between mesenteric or systemic fasting concentration of motilin (mesenteric: 82 $\pm$ 9 pg/ml; systemic: 89 $\pm$ 6; N.S.; Fig. 6A). Thereafter the pigs were fed a standard meal, and blood samples were taken from the cannulas during a 4-h period. Plasma samples were analyzed for motilin and ghrelin using RIA. The mesenteric plasma profiles of the two hormones were notably parallel (Fig. 6B), and linear regression analysis revealed a highly significant linear relationship between the concentration of ghrelin and the concentration of motilin ($r^2 = 0.22$; $P < 0.001$).

Discussion

It is generally accepted that ghrelin is primarily a gut hormone and that ghrelin cells predominate in the oxyntic mucosa of the stomach. However, mapping of ghrelin cell distribution in the GI tract has so far been carefully performed only in rodents (6, 23, 35). In the present study, we performed a thorough mapping of ghrelin cell distribution in the entire human and porcine GI tract. We demonstrate that also the small intestine is a substantial source of ghrelin in humans and pigs. Furthermore, we show for the first time that ghrelin and motilin are produced by the same cells in the small intestine in both species. In addition, we provide evidence for ghrelin and motilin cosecretion from the porcine small intestine.

Within the human stomach, our data show that the ghrelin cells are most frequent in the oxyntic part. In the antrum, on the other hand, ghrelin IR cells were much fewer. These data agree with compiled previous observations (21, 22, 36). It is noteworthy that, for the antrum, the human data contrast to those in rodents, which display relatively high ghrelin cell density also in the antrum (6, 7, 23).

In the duodenum and jejunum of both man and pig, the

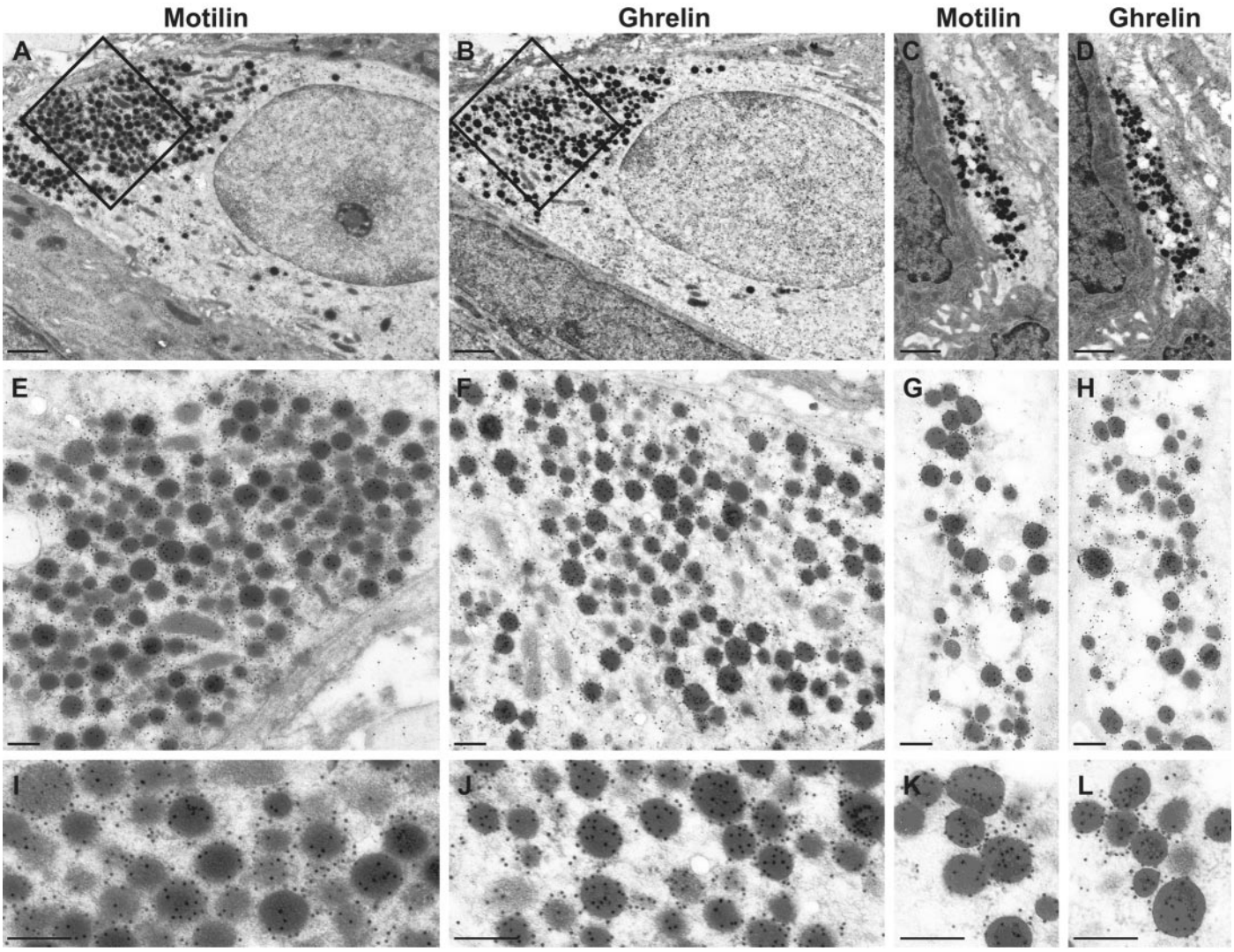

FIG. 4. Human duodenal mucosa. Transmission electron micrographs of cells with immunogold labeling for both motilin (R1105) (A, C, E, G, I, and K) and ghrelin (486–1) (B, D, F, H, J, and L) in consecutive ultrathin sections. Secretory granules from A–D are shown at higher magnification in E–H and even higher magnification in I–L to visualize gold labeling. Panels E and F represent the framed areas in A and B, respectively. Note that gold labeling for both ghrelin and motilin are found in virtually all secretory granules of the same cells. Ghrelin and motilin panels are not superimposable because they are from consecutive section. *Scale bars*, 1 μm (A–D); 250 nm (E–L).

number of ghrelin IR cells was quite high. This finding indicates the existence of a large ghrelin cell pool in the upper small intestine, possibly of comparable size with that in the stomach. The present finding in pig of 3-fold higher ghrelin concentration in the mesenteric vein than in the systemic blood further emphasizes the intestine as a substantial source of circulating ghrelin. These data are supported by findings that humans with short bowel syndrome, due to resection of major portions of the small intestine, display lower plasma ghrelin levels as discussed by Inui *et al.* (37). Furthermore, our present data suggest that the human upper small intestine has higher density of ghrelin cells than that reported for rodents (6, 21, 23, 35). The density of ghrelin cells in the human distal small intestine, colon, and rectum corresponds well with previous observations in rodents (6, 21, 23, 35).

It should be mentioned that the specimens from jejunum were taken from obesity patients operated for weight reduction. The number of ghrelin cells reported here may therefore differ from individuals with normal weight.

We were somewhat surprised to find that the vast majority of the intestinal ghrelin cells were identical with motilin cells in both human and porcine intestine. We feel, however, confident for several reasons that the observed coexistence of the two peptides is not merely due to cross-reactivity of the antisera used. The antisera used were highly specific for ghrelin and motilin, respectively, as illustrated by the fact that the motilin antisera did not stain the ghrelin cells in the stomach, or in the pancreatic islets, in agreement with previous observations (38). In addition, the ghrelin antisera did not stain all the motilin cells. Importantly, ghrelin expression in the human and porcine duodenum and jejunum was confirmed using *in situ* hybridization for ghrelin mRNA. In addition, immunogold labeling for the two peptides was seen in the same duodenal cells using electron microscopy.

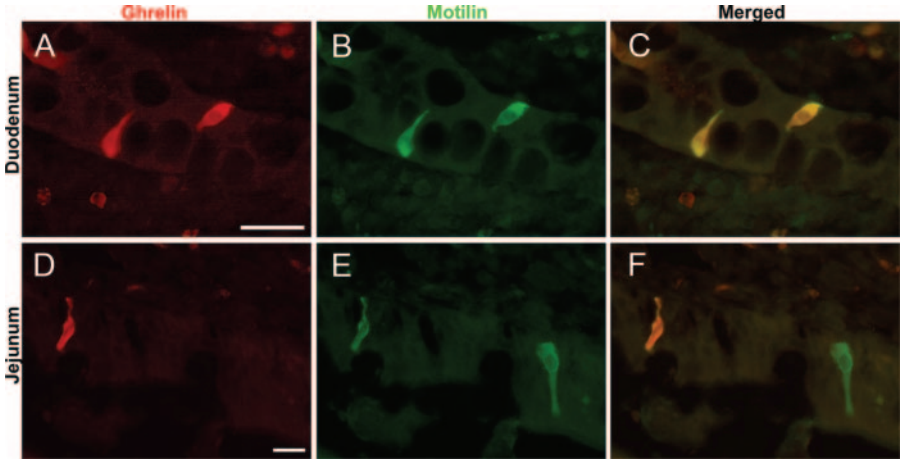

FIG. 5. Porcine small intestine double immunostained for ghrelin (726–2) (*red*, *left*) and motilin (GP1103) (*green*, *middle*); merged (*right*). Ghrelin and motilin are colocalized in the same cells in the duodenum (A–C) and jejunum (D–F). D–F also illustrates an example of a motilin IR cell devoid of ghrelin. *Scale bars*, 50 μm.

Our data on the diameter of the secretory granules of ghrelin/motilin IR cells and the densities of ghrelin/motilin IR cells are in line with previously published data for motilin cells (38, 39). The human and porcine data are also supported by similar findings in monkey, dog, and rabbit (own unpublished observations).

The present finding of both ghrelin IR and motilin IR in the vast majority of the secretory granules of the same cells is a strong evidence for that the two hormones are costored in the same granules. It also suggests that the two hormones are cosecreted from the same cells. These data together with our present findings of parallel plasma profiles of ghrelin and motilin, as well as a correlation between the concentrations of the two hormones in the porcine mesenteric vein, are in strong support of cosecretion of ghrelin and motilin from the same cells in the upper small intestine. Interestingly Schmidt *et al.* (40) demonstrated in systemic human blood that the levels of ghrelin and motilin decreased in parallel postprandially and then increased in parallel to reach preprandial levels. These data are reminiscent of our present data in the porcine portal vein. Together these data suggest that ghrelin and motilin are cosecreted from ghrelin/motilin IR cells also from the human intestine.

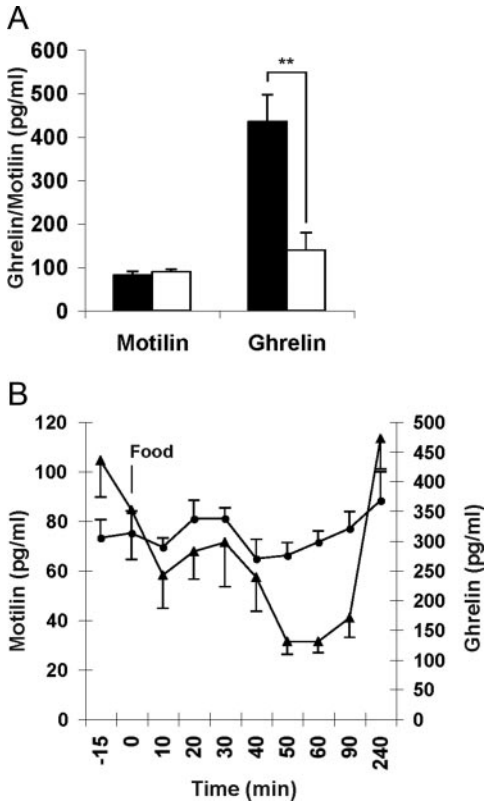

FIG. 6. A, Fasting concentrations of ghrelin and motilin in mesenteric (*black bars*) and systemic (*white bars*) plasma. B, Plasma concentration of ghrelin (*triangles*) and motilin (*circles*) in the porcine superior mesenteric vein (sampled distal to the junction with the gastroepiploic vein) after a standard meal. Note that the plasma profiles of the two hormones are parallel. Data presented as mean ± SEM (n = 6).

It may be mentioned that we did not find any difference between fasting levels of motilin in mesenteric compared with systemic blood. One explanation for this could be that motilin may have a higher turnover rate in the circulation than ghrelin. Another possibility is that sources other than the GI tract of circulating motilin exist. One such potential source is the thyroid, in which motilin is produced in certain species (41, 42).

Motilin has been regarded as the initiator of the interdigestive peristaltic reflex, the migrating motor complex (MMC), in the GI tract because increased motilin levels have been measured concomitant with the phase III activity of MMC in the intestine (43–47). It should also be pointed out that motilin, like ghrelin, but unlike many other intestinal hormones, is not secreted postprandially (5). This seems rational because both hormones stimulate MMC, which is needed first when the meal is digested. Because ghrelin is also prokinetic (reviewed in Ref. 3), it may be as important as motilin in this respect. Animal experiments have shown stimulating effects of ghrelin on gastric emptying and intestinal motility (8–14). Recent data indicate that ghrelin accelerates gastric emptying also in man (18, 19), and plasma ghrelin levels were found to correlate positively with gastric emptying rate (5) and bowel movement frequency (48). Furthermore, ghrelin has been shown to initiate premature phase III of the MMC, not mediated by release of motilin (17). Ghrelin was also shown to increase gastric emptying independent of motilin (20). It remains to be established whether the motility effects of ghrelin are caused mainly by activation

of central efferents, vagal afferents, or the enteric nervous system (3). Our present finding of colocalization of ghrelin and motilin in the duodenal and jejunal mucosa is intriguing in view of the role these peptides have as regulators of gastric and intestinal motility. These roles are to some extent exerted independently (17), but the present finding of IR for both ghrelin and motilin in the secretory granules of the same cells, together with our data, suggesting cosecretion of the two hormones, may foster studies on the possibility of concerted actions of the two peptides. Furthermore, the intestinal ghrelin and motilin coexistence may have pathophysiological implications for, for example, dysmotility and bariatric surgery.

We conclude that the duodenum and jejunum harbor a considerable population of ghrelin cells. This raises the possibility of important physiological functions of intestinal ghrelin. The vast majority of the intestinal ghrelin cells are identical with motilin cells, and the two hormones are costored and cosecreted. The possibility that ghrelin acts in concert with motilin in the regulation of motility needs further investigation.

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