Duodenal Leptin Stimulates Cholecystokinin Secretion
Evidence of a Positive Leptin-Cholecystokinin Feedback Loop
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Some of the actions of leptin depend on cholecystokinin (CCK). However, it is unknown whether leptin modulates the release of CCK. Here, we demonstrate in vitro that leptin induces the phosphorylation of extracellular signal-related kinase (ERK)-1/2 proteins and increases CCK release (EC50 = 0.23 nmol/l) in CCK-secreting STC-1 cells. We showed that rat duodenal juice contains leptin that circulates free and bound to macromolecules, suggesting that leptin has a luminal action on the intestine. In vivo in the rat, duodenal infusion of leptin increased plasma CCK at levels comparable to those induced by feeding. Moreover, meal-induced increases in plasma CCK were markedly reduced in obese fa/fa rats, whereas the mobilization of the gastric leptin pool was similar in lean and obese Zucker rats. The release of CCK by leptin presumably generates a positive feedback loop. Indeed, the blockade of CCK receptors reversed the meal reduction of the stomach leptin pool and the meal-increased plasma insulin, consistent with the previous concept of an entero-insular axis. Collectively, these data support a novel mode of action of leptin involving cross-talk between hypothalamic leptin receptors and various neuropeptides involved in the control of feeding. The leptin receptor (Ob-R) is a member of the gp130 family of cytokine receptors. It occurs in several isoforms resulting from the alternative splicing of the db leptin receptor gene (2,3). It is currently thought that the long isoform, Ob-Rb, can activate the signal transducers and activators of transcription (STAT) pathways, whereas both Ob-Rb and the short isoform (Ob-Ra) can transduce signals through insulin receptor substrates and through mitogen-activated protein kinase (MAPK) pathways (7).

The signals that arise from the upper gastrointestinal tract upon feeding are transmitted to the brain by the vagus nerve. These signals are key components in the control of meal-induced satiety. Cholecystokinin (CCK) is secreted from duodenal endocrine I cells and typically functions as one of these short-term satiety signals (8,9). Interestingly, the leptin-induced inhibition of food intake (10) and the stimulation of pancreatic exocrine secretions (11) can be blocked by a CCK-1 receptor antagonist. These data suggest that endogenous CCK is involved in these effects, operating through CCK-1 receptors. However, it is not currently known whether leptin directly modulates the release of CCK.

Leptin is also produced by the stomach (12–14) and is mainly secreted into the gastric juice after CCK in rats (12,15) and after secretin or vagal stimulation in humans (14,16). Some of the stomach-derived leptin is not fully degraded by proteolysis, indicating that it reaches the intestine in an active form and thus can initiate biological processes controlling functions of the intestinal tract. Indeed, luminal leptin increases the activity of the brush border proton-dependent transporter, PepT1, which enhances the intestinal absorption of oligopeptides (17). This raises the possibility that leptin could also modulate the secretory activity of intestinal endocrine cells, provided that leptin is present in the intestinal juice.

In this study, we examined the in vitro effects of recombinant leptin on the release of CCK and investigated the intracellular mechanisms of leptin action in CCK-secreting STC-1 cells. We hypothesized that leptin that reaches the duodenum is able to modulate the release of CCK. To test this hypothesis, we determined whether leptin was present in the duodenal juice and then examined the in vivo effect of direct duodenal delivery of leptin on plasma CCK concentrations in the rat duodenal-perfused model. We questioned the physiological relevance of the data by analyzing the in vivo kinetic patterns of plasma CCK and insulin concentrations and by analyzing the leptin content of the stomach after food intake in Zucker rats. Finally, we investigated the in vivo effects of
**RESEARCH DESIGN AND METHODS**

**Cell culture.** STC-1 cells (a gift from Dr. J. Abello, the Institut National de la Santé et de la Recherche Médicale [INSERM] U-45, Lyon, France) are derived from intestinal endocrine tumor cell lines developed from mice expressing the transgene for the rat insulin promoter linked to the simian virus 40 large T antigen and the polyoma virus small t antigen (18). STC-1 cells between passage 15 and 35 were grown in RPMI-1640 plus glutamine (Sigma, St. Louis, MO), supplemented with 5% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Grand Island, NY) in a humified atmosphere containing 5% CO₂ and 5% O₂ at 37°C.

**RT-PCR analysis of leptin receptors.** Total RNA was extracted from STC-1 cells with the Trizol reagent (Invitrogen, Carlsbad, CA). Briefly, the first-strand cDNA was synthesized from 2 μg total RNA and was reverse-transcribed with 200 units of reverse transcriptase using the Superscript II kit (Invitrogen) according to the manufacturer's recommendations. The following oligonucleotides primers were synthesized by Sigma Genosys (Cambridgehire, U.K.): the forward primer for Ob-Rb was 5'-ATGAACTGTTGATGACCCCTTCG-3' and the reverse primer was 5'-ATATACGTCTTGGATCTGCTGG-3'. The primers used for the β-actin gene were as follows forward 5'-CCAGAAGATGACCCCATGATG-3' and reverse 5'-ATGATCTCCTTGTGACCTG-3'. Samples were denatured by heating at 95°C for 5 min. PCR was then carried out under the conditions previously described (14). PCR products were separated by electrophoresis in a 2% agarose gel. The gel was stained with ethidium bromide and viewed under ultraviolet illumination. The expected sizes of the PCR products were 375 bp for Ob-Rb and 606 bp for β-actin.

**Western blot analysis.** For total protein extraction, STC-1 cell pellets were homogenized at 4°C in lysis buffer supplemented with 0.1 mg/ml phenylmethylsulfonyl fluoride, 100 μM dithiothreitol, and 100 μM NaVO₄. The homogenates were centrifuged at 15,000 × g for 10 min at 4°C, and the supernatants were collected for Western blot analysis. Protein concentration was quantified using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

**Immunocytochemistry.** STC-1 cells were seeded into 4-well Lab-Tek II glass slides (Nalge Nunc International, Naperville, IL). After 2 days of incubation at 37°C with 95% O₂, 5% CO₂, the medium was removed and the cells were washed twice in cold PBS. We then added a formalin fixative (4% paraformaldehyde, in PBS) to the cells for 10 min, which were then digested with 0.1% Tris-buffered saline (0.1% HCl, pH 2.25, for 10 min to remove the antigen. Before immunoreaction, endogenous peroxidase activity was removed by adding 3% H₂O₂ and incubating for 10 min. The cells were incubated overnight at 4°C with a rabbit polyclonal Ob-Rb antisera (OBR D2-A) or polyclonal Ob-R receptor (amino acids 890–903) specific to the Ob-Rb isoform (OBR D12-A; Biotrend Chemikalien, Cologne, Germany) diluted 1:750. After a 1-h incubation of the membranes with anti-rabbit horseradish peroxidase–conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:1,000, the immune complexes were detected by enhanced chemiluminescence (Pierce, Rockford, IL).

**Determination of leptin in duodenal juice and size exclusion chromatography.** Rats fed ad libitum or rats that had been deprived of food for 24 h were anesthetized, and an outflow cannula was surgically implanted 1 cm below the ligament of Treitz for the collection of duodenal juice for 60 min after intraperitoneal injection (through the femoral vein) of saline or 30 μg/kg of carbachol (Sigma, St. Louis, MO). The pH was measured, and leptin was determined by RIA. A sample of duodenal juice or murine leptin was submitted to size exclusion chromatography using Superdex 200 column (16/60) (Pharmacia Biotech, Freiburg, Germany). The column was equilibrated with PBS containing 0.1% BSA and 0.01% sodium azide at 4°C at a flow rate of 2 ml/min. The applied sample volume was 5 ml, and fractions of 2 ml were collected and stored at −20°C until leptin RIA. The column was adjusted with commercially available calibration proteins kits (Pharmacia Biotech).

**Duodenal perfusion studies.** Duodenal perfusion studies were carried out on rats that had been deprived of food for 24 h, with water available ad libitum. The rats were anesthetized with ethylurethane (1.2 g/kg, intramuscularly) (Prolabo, Paris, France), and after laparotomy, a transpyloric infundibulum cannula was inserted into the duodenum and an outflow cannula was inserted ~1 cm below the ligament of Treitz. The duodenal segment was perfused at a flow rate of 4 ml every 15 min with a Krebs-Ringer buffer consisting of the following (in mmol/L): 0.5 MgCl₂, 4.5 KCl, 120 NaCl, 0.7 NaHPO₄, 1.5 NaH₂PO₄, 1.2 CaCl₂, 15 NaHCO₃, and 10 glucose adjusted to pH 7.5. The solution was maintained at 37°C by a water-jacket before its entry into the duodenal segment. After a 15-min stabilization period, vehicle containing BSA as a nonspecific protein or murine leptin (1–100 μmol/L) was added in the perfusion solution. Blood samples were collected through a carotid catheter in tubes containing EDTA as previously described (11) and were centrifuged at 10,000g for 3 min. Plasma was removed and speedvac concentrated after an ethanol extraction and stored at −20°C until CCK RIA.

**Effects of feeding alone or in association with CCK receptor antagonists on stomach leptin, plasma insulin, and CCK levels.** Male Wistar or Zucker rats were habituated to eat their pellet diets between 10:00 A.M. and 6:00 P.M. when food was withdrawn until the next morning, but water was available ad libitum.

**Antagonists.** The day of the experiment starting in the morning (10:00 A.M.), the animals were allowed to feed on preweighed standard laboratory pellet diet for different time periods. At each period, food was weighed and intake was determined. The rats were anesthetized, blood was collected from the abdominal aorta and centrifuged, and plasma was removed and stored at −20°C until RIA of CCK and insulin (RIA kits; Linco Research, St. Charles, Mo.). Plasma CCK was then assayed. Its concentration was determined by RIA, and the immunoreactive hormone and the supernatants were removed and stored at −20°C until assayed for leptin in gastric juice by RIA. The fundic mucosa was scraped off, weighed, homogenized, and centrifuged 10,000g for 10 min as previously described. The supernatants were used for the determination of stomach leptin.

**CCK receptor antagonists.** For the antagonist studies, 10 min before food intake, the animals were intraperitoneally injected with vehicle (control) or 1 mg/kg L654718 (a gift from Professor B. Roques, INSERM 266, Paris, France) or YM022 (a gift from MAPK (pTyr185/187) phosphospecific (Biosource Europe, Nivelles, Belgium) diluted 1:1,000. The same membranes were then stripped and immunoblotted with an ERK-1 antisera (Santa Cruz Biotechnology) diluted 1:500 to determine total MAPK proteins. The immune complexes were detected by enhanced chemiluminescence (Pierce). The blots were probed overnight at 4°C with rabbit polyclonal antibodies: anti-ERK-1/2.
Yamanouchi Pharmaceutical, Tokyo), CCK-1 and CCK-2 receptor antagonists, respectively, in saline containing 1% DMSO. After 15 min of food intake, plasma insulin, plasma CCK, stomach leptin content, and leptin release were determined.

RIA of CCK. CCK was determined according to the procedure described by Rehfeld (19). Briefly, the COOH-terminal anti-CCK antibody (a gift from Professor J. Rehfeld, Copenhagen, Denmark) was incubated at 4°C for 4 days with CCK-8 (Sigma) or with plasma samples and [125]I-CCK-8 (Amersham Pharmacia Biotech, Piscataway, NJ) in RIA buffer consisting of 20 mmol/l barbital buffer, 0.6 mmol/l thiomersal, and 0.11% BSA vol/vol (pH 8.4). Bound and free fractions were separated by absorbing the free [125]I-CCK-8 onto active dextran T70-coated charcoal (4 and 40 gl, respectively) in RIA buffer containing 10% filtered horse serum. Radioactivity in the bound fraction was measured with a gamma counter. Under these conditions, the detection limit was 0.5 pg CCK.

**Statistical analysis.** The results are expressed as means ± SE. They were compared by one-way ANOVA, followed by a Tukey-Kramer multiple comparisons test if significant results were obtained.

**RESULTS**

**STC-1 cells express leptin receptors.** RT-PCR and Western blot analysis were used to study the expression of the leptin receptors in CCK-producing STC-1 cells. A 375-bp product, corresponding to positions 2401–2776 of Ob-Rb, was detected (Fig. 1A). After cDNA sequencing, this product was found to be 100% identical to the mouse Ob-Rb gene transcript.

Immunoblotting of STC-1 protein extracts with an anti-Ob-Rb antibody detected two immunoreactive (IR) bands with relative molecular masses of 130- and 170-kDa (Fig. 1B). The prominent 130-kDa IR band corresponded to the long isoform of the leptin receptor based on the predicted molecular weight, and the 170-kDa band probably corresponded to a glycosylated form of Ob-Rb. Immunocytochemical studies (Fig. 1C and D) using an antibody that recognizes all leptin receptor isoforms showed leptin receptor immunoreactivity diffusely distributed in the cytoplasm with strong staining on the membranes of some cells (Fig. 1C). The use of an antiserum specific for Ob-Rb gave a similar distribution of leptin receptors in STC-1 cells (Fig. 1D).

**Leptin stimulates the release of CCK from STC-1 cells.** Immunostaining of the STC-1 cells with a specific anti-CCK antibody showed that 90% of the cells contained CCK (data not shown), which is consistent with the results of previous studies (20). The mean cellular CCK content was 1.308 ± 101.5 pmol/l (n = 16), and the basal level of CCK release over 1 h (24.9 ± 6.2 pmol/l, n = 16) accounted for 1.9% of the total CCK content of the cells.

Consistent with the results of previous studies (21,22), the neuropeptide bombesin (used as a positive control) stimulated the release of CCK in a concentration-dependent manner; 100 nmol/l stimulated an increase of 229.0 ± 29.9% from the basal levels (P < 0.05 vs. basal) (Fig. 2A). The addition of leptin to STC-1 cells also induced a concentration-dependent increase in CCK release. Stimulation began to occur at a leptin concentration of 0.01 nmol/l (114 ± 10.9% of basal) and became significant at a leptin concentration of 0.1 nmol/l (130.6 ± 9.2% of basal; P < 0.05). Maximal leptin stimulation (188.1 ± 19.2% of basal, P < 0.01) was achieved with 10 nmol/l leptin, and no further increase was observed with 100 nmol/l leptin. This maximal effect corresponded to 80% of that induced by bombesin (100 nmol/l). The concentration of leptin producing a half-maximal stimulation (EC50) of CCK release was 0.23 ± 0.08 nmol/l. The leptin-stimulated CCK release was associated with a significant decrease in total cellular CCK content; a 40% decrease was observed with 10 nmol/l leptin (Fig. 2B).

**Leptin induces ERK phosphorylation and PD98059 inhibits leptin-stimulated CCK release.** Immunoblotting of STC-1 cell extracts with an anti-ERK antiserum specific for the long isoform (Ob-Rb) of the leptin receptor gene. **Lane 1:** marker DNA ladder; lane 2: STC-1 cells; lane 3: negative control in which the reverse transcriptase was omitted; lane 4: β-actin. The expected sizes of the PCR products were 375 bp for Ob-Rb and 606 bp for β-actin (arrows). **B:** Western blot analysis of STC-1 cell extracts at passage 15 (lane 1), 25 (lane 2), and 35 (lane 3), with a specific anti-Ob-Rb antibody (OBR 12-A). A representative immunoblot is shown for the leptin receptor, showing two IR proteins, 130 and 170 kDa in size. Note that the amount of leptin receptor protein did not change from passage 15–35. **C** and **D:** STC-1 cells were fixed in 4% paraformaldehyde. Immunostaining of leptin receptors was performed with two different rabbit polyclonal antibodies: TP 283 (C), which recognizes all Ob-R isoforms, and OBR 12-A (D), an antiserum specific for the Ob-Rb isoform. Arrows indicate strong signals on STC-1 cell membranes.
fected by wortmannin at concentrations up to 10 μmol/l (Fig. 3B). These data indicate that the activation of ERK proteins contributes to the stimulatory effect of leptin on the release of CCK from STC-1 cells.

**Duodenal leptin increases plasma CCK levels in vivo.**

To verify our hypothesis that gastric leptin that enters the duodenum modulates intestinal biological processes, we examined the occurrence of leptin in the duodenal juice. The duodenal juice contained high amounts of leptin-IR proteins (Fig. 4A). In basal conditions, these amounts were higher in rats fed ad libitum than in fasted rats (146 ± 9 ng leptin/ml \([n = 7]\) vs. 92 ± 11 ng leptin/ml \([n = 14]\); \(P < 0.05\)). Furthermore, intravenous injection of the cholinergic muscarinic agonist carbachol resulted in a significant increase in duodenal leptin-IR in fasted rats. The elution profile of the basal duodenal juice after size exclusion chromatography showed two leptin IR peaks: peak I corresponded to leptin bound to macromolecules and peak II corresponded to murine leptin and eluted at the expected molecular weight of 16 kDa (Fig. 4B). The molecular weight of peak I ranged from the void volume at 430 to 232 kDa with a maximum at 300 kDa. These data indicate that leptin circulates in the duodenum juice both free and bound to high–molecular weight proteins.

We then examined the effect of delivering leptin directly into the duodenum on plasma CCK concentrations. The basal plasma CCK levels were higher in ad libitum–fed rats (0.9 ± 0.2 pmol/l, \(n = 7\)) than in fasted rats (0.42 ± 0.1 pmol/l). Intraduodenal infusion of leptin induced a concentration-dependent increase of basal CCK levels in fasted rats (Fig. 4C). Leptin (1 nmol/l) did not significantly modify plasma CCK levels. However, a significant increase was observed for 10 nmol/l leptin (2.1 ± 0.1 pmol/l at 15 min). A further increase was observed with 100 nmol/l leptin with values of 5.01 ± 1.2 pmol/l after 15 min and a
peak value of 6.02 ± 1.8 pmol/l (P < 0.01) 30 min after
infusion; after 60 min, values then progressively decreased
to 1.25 ± 0.6 pmol/l after 60 min (Fig. 4C).

**Feeding decreases the gastric pool of leptin and increases plasma CCK levels.** To study the physiological relevance of these results, we analyzed the kinetic patterns of plasma CCK and insulin concentrations in parallel with changes in stomach leptin content and release after food intake.

Feeding induced a rapid (as early as 15 min) and
marked decrease (70%, P < 0.01 vs. control) in stomach content of leptin and a parallel 3.5-fold increase in leptin
release into the gastric juice (Fig. 5A). This effect was
associated with a significant increase in the plasma insulin concentration (positive control) that peaked after 15 min
(7.8 ± 1.1 vs. 0.9 ± 0.3 ng/ml; P < 0.01 vs. control). In
addition, the basal plasma CCK concentration also in-
creased upon feeding, reaching a peak of 4.42 ± 0.86
pmol/l after 30 min and then slightly decreasing to a value
of 3.6 ± 0.73 pmol/l after 60 min (Fig. 5B).

These kinetic patterns were further analyzed in the
Zucker rat. In lean *Fa/fa* and in obese *fa/fa* rats, feeding
markedly decreased the gastric leptin content (50% at
15 min, P < 0.01 vs. control) (Fig. 6A) and increased the
amount of leptin secreted into the gastric juice (data not
shown). However, the plasma CCK response to a meal was
impaired in obese *fa/fa* rats. The basal plasma CCK con-
centration of lean *Fa/fa* rats was not significantly different
from that of obese *fa/fa* rats (0.36 ± 0.08 vs. 0.29 ± 0.1
pmol/l) (Fig. 6B). In lean *Fa/fa* rats, feeding increased plasma CCK concentrations by up to eightfold, whereas in
obese *fa/fa* rats, the plasma CCK concentrations increased
less dramatically in response to a meal (50%, P < 0.01 vs.
lean *Fa/fa*). It should be noted that there was no significant
difference between the amounts of food consumed by lean
*Fa/fa* rats and obese *fa/fa* rats (2.2 ± 0.2 vs. 2.7 ± 0.5 g).

**CCK receptor antagonists abolish feeding-induced changes in stomach leptin and plasma insulin.** To determine whether a feedback loop exists between endoge-
 nous CCK and gastric leptin in physiological conditions, we
investigated the effects of CCK receptor antagonists. The
prior injection of L364718 or YM022 inhibited the
feeding-induced increase in plasma insulin concentration
by 68% (P < 0.001 vs. control) (Fig. 7A) and did not affect
postprandial plasma CCK concentrations (Fig. 7B). In
these conditions, a complete reversion of the reduction of
the stomach content of leptin and the increase in gastric
leptin release after food intake was observed (Fig. 7C and
D). Moreover, a trend toward increased food intake was
observed after CCK receptor antagonists (2.7 ± 0.4 g for
YM022; 2.3 ± 0.5 g for L364718 vs. 1.6 ± 0.4 g for control).
These data indicate that endogenous CCK via activation of
CCK receptors is involved in the control of gastric leptin as
well as insulin secretion.
DISCUSSION

In this report, we provide evidence that the long isoform of the leptin receptor (Ob-Rb) is expressed in CCK-producing STC-1 cells. These leptin receptors were diffusely distributed in the cytoplasm and were detected on the plasma membrane of STC-1 cells. The expression of leptin receptors in STC-1 cells makes this cell line a suitable model for the investigation of leptin physiology. Thus, we demonstrated that the incubation of STC-1 cells with leptin increases the amount of CCK released and decreases the total CCK cell content, suggesting that a preformed pool of CCK was mobilized.

It is now well established that only the Ob-Rb isoform activates the STAT pathways, whereas both the long (Ob-Rb) and short (Ob-Ra) isoforms can transduce signals through insulin receptor substrates and the MAPK cascade. Our data indicate that MAPK-dependent pathways are actually operating in STC-1 cells for the leptin-stimulated secretion of CCK. Indeed, leptin induces a rapid phosphorylation of ERK-1/2 proteins, in agreement with the failure of leptin to affect the amount of CCK released from STC-1 cells when the ERK activators MEK-1/2 were pharmacologically inhibited. A similar MAPK-dependent pathway has been reported for bombesin-induced increases in CCK release in the same model (22). The downstream events that follow the activation of MAPK and lead to CCK secretion are unknown. STC-1 cells express the three synaptic core complex proteins syntaxin-1, synaptosomal-associated protein-25 (SNAP-25), and vesicle-associated membrane protein 2, which plays a key role in vesicle exocytosis (23). Moreover, the activation of MAPK is associated with an increase in the phosphorylation of synapsin I in neuronal preparations (24,25). Therefore, it is possible that, in our conditions, leptin facilitates the exocytosis of secretory vesicles containing CCK; however, this remains to be demonstrated.

Because these STC-1 cells do not closely represent duodenal I cells, the in vivo relevance of these data was analyzed in the rat. In particular, we verified our hypothesis that some of the leptin originating from the stomach reaches the duodenum in an active form, thereby modulating the release of CCK. This result implies, however, that leptin is present in the duodenal juice. Thus, we demonstrated that duodenal juice contains high amounts of leptin that circulates both free and bound to high–molecular weight proteins. These leptin-binding proteins have been shown to be the soluble leptin receptor in the blood (26). Although the precise nature of the binding protein interacting with leptin in the duodenal juice requires further elucidation, it is possible that the ratio of

FIG. 5. Feeding decreases stomach leptin stores and increases plasma CCK and insulin levels. A: Changes in fundic mucosa leptin and in the amount of leptin released in the gastric juice in Wistar rats that were fasted overnight and fed for the indicated time periods. Data are expressed as the mean ± 1 SE of n = 8. B: Plasma CCK and insulin response to food intake in Wistar rats. Data are expressed as plasma CCK levels in picomoles per liter, and each point corresponds to the means ± SE of eight rats in each group. *P < 0.05; **P < 0.01 vs. time 0.

FIG. 6. Feeding decreases stomach leptin stores in Zucker rats. A: Changes in fundic mucosa leptin in male Zucker rats that were fasted overnight and fed for the indicated time periods. Data are expressed as the mean ± 1 SE of n = 9. B: Plasma CCK response to food intake in male Zucker rats. Data are expressed as the mean ± 1 SE of n = 9 for lean Fa/fa and obese fa/fa rats. *P < 0.05; **P < 0.01 vs. time 0; ##P < 0.01 vs. lean Fa/fa.
free to bound leptin may be affected by the metabolic status. Thus, this unknown leptin-binding protein may have physiological implications in terms of the bioavailability of leptin and might help us to understand the action of leptin on the intestinal epithelium. The presence of leptin immunoreactivity in duodenal juice, along with the previous data reporting the expression of the leptin receptor on the apical side of the enterocytes (17,27), make it likely that leptin exerts a lumenal action on the intestinal epithelium.

As expected, the direct infusion of leptin into the duodenum increased plasma CCK at levels comparable to those induced by feeding, suggesting that leptin regulates the secretory activity of the endocrine I cells. In response to food intake, both lean Fa/fa and obese fa/fa rats exhibited a similar decrease in gastric leptin content and an increase in the amount of leptin released in the gastric juice. This result suggests that the meal-regulated pathways of gastric leptin are functional in these rats and may also indicate that similar amounts of released gastric leptin reach the duodenum in both groups of rats. However, the plasma CCK response to a meal is markedly reduced in obese fa/fa rats compared with lean Fa/fa rats. The genetically obese fa/fa rats carry a missense mutation that results in an amino acid substitution at position 269 (Gln → Pro) within the extracellular domain of the leptin receptor (28) and leads to a strong decrease of function of the receptor (29). Therefore, a decreased functional efficiency of the leptin receptor may account for the loss of sensitivity of fa/fa rats to meal-stimulated plasma CCK. Similar data showing a reduced CCK release in the hypothalamus of fa/fa rats in response to a meal have been reported (30). Taken together, it seems that functional leptin pathways are required for full plasma CCK response to a meal. However, whether leptin in duodenal fluid directly affects meal-induced CCK release requires further studies.

The in vivo effective dose of leptin in this study is consistent with that detected in the duodenal juice in rats fed ad libitum (9.2 nmol/l) compared with 5.5 nmol/l in fasted rats under basal conditions. These data strongly suggest the involvement of meal regulatory processes in the changes of leptin in duodenal fluid in these animals. This led us to conclude that, in physiological conditions, the rapid mobilization of the gastric leptin pool that enters the intestine is involved in the release of CCK from the duodenal endocrine I cells. It is noteworthy that the amount of leptin in basal duodenal juice is greater than the amount presumed to originate from the stomach. This intriguing but interesting finding can be tentatively explained by a contribution from other leptin-producing sites that remain to be identified. In addition to gastric secretions, the duodenum receives hepato-biliary and exocrine pancreatic secretions. Whether these secretions contain leptin that can contribute to the increase in leptin concentrations in the duodenal juice is currently under investigation.

The data presented here may have physiological implications. Previous studies have shown that CCK-1 receptor antagonists can prevent the peripheral leptin-induced inhibition of food intake (10) and the stimulation of pancreatic exocrine secretions (11), which suggests that CCK is involved. However, whether leptin modulates the release of CCK is unknown. Our findings that duodenal leptin increases the amount of CCK released provide a basis for the explanation of these CCK-dependent effects of leptin.
Whereas these data support a model in which meal-induced leptin release into duodenal fluid contributes to prandial CCK release, the contribution of this effect, relative to that of known CCK secretagogues (e.g., ingested nutrients), remains unknown and requires additional study. Conversely, the presence of leptin circulating in the duodenum, which also contains hepato-biliary secretions, could be important for the elimination of biliary cholesterol by regulating the enterohepatic circulation of bile salts, as recently reported (31).

The release of CCK by leptin presumably generates a positive feedback loop, because, as we have previously reported, CCK stimulates the release of gastric leptin (12). This is likely because the blockade of CCK receptors completely prevents the decrease in stomach leptin content as well as the rise in plasma insulin concentrations in response to a meal. These effects are associated with trends toward increased food intake, suggesting that during normal digestion, this positive feedback loop may be operational in the reduction of food intake. These data also support the concept of an entero-insular axis, in which CCK may regulate postprandial insulin concentrations (32,33). They do not, however, exclude the involvement of other regulatory peptides in meal-induced insulin release.

In summary, this study demonstrates that leptin, via a luminal mode of action, increases plasma CCK concentration through the leptin receptor probably linked to MAPK-dependent pathways. It supports a novel mode of action of leptin where, in physiological conditions, leptin and CCK may potentiate their own actions by cross-stimulating their secretion. The impairment of this leptin-CCK loop may have pathological implications related to obesity and diabetes.

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