Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men

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Degen L, Drewe J, Piccoli F, Gräni K, Oesch S, Bunea R, D’Amato M, Beglinger C. Effect of CCK-1 receptor blockade on ghrelin and PYY secretion in men. Am J Physiol Regul Integr Comp Physiol 292: R1391–R1399, 2007. First published November 30, 2006; doi:10.1152/ajpregu.00734.2006.—Cholecystokinin (CCK), peptide YY (PYY), and ghrelin have been proposed to act as satiety hormones. CCK and PYY are stimulated during meal intake by the presence of nutrients in the small intestine, especially fat, whereas ghrelin is inhibited by eating. The sequence of events (fat intake followed by fat hydrolysis and CCK release) suggests that this process is crucial for triggering the effects. The aim of this study was therefore to investigate whether CCK mediated the effect of intraduodenal (ID) fat on ghrelin secretion and PYY release via CCK-1 receptors. Thirty-six male volunteers were studied in three consecutive, randomized, double-blind, cross-over studies: 1) 12 subjects received an ID fat infusion with or without 120 mg orlistat, an irreversible inhibitor of gastrointestinal lipases, compared with vehicle; 2) 12 subjects received ID long-chain fatty acids (LCF), ID medium-chain fatty acids (MCF), or ID vehicle; and 3) 12 subjects received ID LCF with and without the CCK-1 receptor antagonist dexloxiclumide (Dexloxo) or ID vehicle plus intravenous saline (placebo). ID infusions were given for 180 min. The effects of these treatments on ghrelin concentrations and PYY release were quantified. Plasma hormone concentrations were measured in regular intervals by specific RIA systems. We found the following results. 1) ID fat induced a significant inhibition in ghrelin levels (P < 0.01) and a significant increase in PYY concentrations (P < 0.004). Inhibition of fat hydrolysis by orlistat abolished both effects. 2) LCF significantly inhibited ghrelin levels (P < 0.02) and stimulated PYY release (P < 0.008), whereas MCF were ineffective compared with controls. 3) Dexloxo administration abolished the effect of LCF on ghrelin and on PYY. ID fat or LCF significantly stimulated plasma CCK (P < 0.006 and P < 0.004) compared with saline. MCF did not stimulate plasma CCK release. In summary, fat hydrolysis is essential to induce effects on ghrelin and PYY through the generation of LCF, whereas MCF are ineffective. Furthermore, LCF stimulated plasma CCK release, suggesting that peripheral CCK is the mediator of these actions. The CCK-1 receptor antagonist Dexloxo abolished the effect of ID LCF, on both ghrelin and PYY. Generation of LCF through hydrolysis of fat is a critical step for fat-induced inhibition of ghrelin and stimulation of PYY in humans; the signal is mediated via CCK release and CCK-1 receptors. peptide YY; cholecystokinin

THE PROCESS OF FOOD INTAKE is coordinated by interactions between the enteric nervous system and gastrointestinal hormones (34, 36). Many gastrointestinal hormones are released from specialized endocrine cells into the circulation or serve as neurotransmitters that mediate signals from the enteric nervous system. These signals are transmitted from the gut to the brain and become integrated at various centers in the hypothalamus, reflecting the load of nutrients ingested (34). As a group, these peptides are called satiety signals because most create a sensation of fullness and reduce food intake when administered to humans or animals.

Cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and ghrelin are examples of gastrointestinal signals that exert various physiological effects in the gastrointestinal tract, including modulation of appetite and satiety (4, 17, 35). CCK, GLP-1, and PYY exert inhibitory effects (4, 10, 11, 17, 22) on food intake and satiety, whereas ghrelin initiates meal ingestion (10, 11). CCK, GLP-1, and PYY are stimulated from specialized endocrine cells into the circulation during meals by the presence of food in the intestine; in contrast, ghrelin secretion is increased by fasting and inhibited by meal ingestion.

Our group and others have previously established that fat digestion is crucial for stimulation of CCK release (13, 21, 30, 31); 1) triglycerides infused to the small intestine are a powerful stimulus for CCK release; 2) intestinal infusion of the lipase inhibitor orlistat together with triglycerides virtually abolishes the postprandial release of CCK and markedly reduces exocrine pancreatic secretion; 3) finally, only long-chain fatty acids (LCF) are able to induce CCK release and inhibit food intake, whereas medium-chain fatty acids (MCF) are ineffective.

In addition, it has been shown that intraduodenal fat can inhibit ghrelin secretion (12–15) and that fat hydrolysis is essential for inducing this effect. We thus hypothesized that the effect of hydrolyzed fat is mediated by CCK release via CCK-1 receptors. The aim of this study was therefore to investigate whether CCK mediated the effect of intraduodenal fat on ghrelin secretion via CCK-1 receptors. We were also interested to evaluate the role of CCK in regulating PYY secretion; PYY is produced in L cells from the gut on stimulation with fat.

METHODS

Subjects

Thirty-six male subjects, aged 20–30 yr (mean 25.2 yr), participated in the study. Body weight of all subjects was within the normal range for age, sex, and height. Each subject gave written, informed consent for the study. The human ethics committee of the University Hospital, Basel, approved the protocol. Before acceptance, each participant was required to complete a medical interview, receive a full physical examination, and participate in an initial laboratory screening. No subject was receiving any medications or had a history of food allergies or dietary restrictions.

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Experimental Procedure

Part I: effect of orlistat dissolved in olive oil on hormone secretions. Three treatments, separated by at least 7 days, were performed in 12 subjects in a randomized order. On the evening preceding each treatment, subjects swallowed a radiopaque polyvinyl feeding tube (external diameter 8 Fr), which had an opening at the tip of the tube. The tube was inserted through the nose because this procedure allowed the tube to be retained overnight and for the duration of the experiment but also allowed subjects to eat and drink with minimum discomfort. The tube was transported to the duodenum overnight. In the morning, the position of the tube was located fluoroscopically, and the tip of the tube was positioned 100 cm distal to the teeth. It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment.

The treatments were identical in design except for the intraduodenal infusions. One treatment consisted of intraduodenal saline infusion for the duration of the experiment (180 min). In the second and third experiments, intraduodenal fats (olive oil) with or without 120 mg tetrahydrolipstatin was used instead of saline throughout the experiment. An infusion rate of 0.5 ml/min oil (load 41 g) was chosen for the duration of the experiment; this rate was taken from previous experiments (21, 29, 30). The intraduodenal fat infusion solution was indistinguishable in appearance from the control solution (saline). The treatments were given in a double-blind manner. During the experiments, samples of blood (7.5 ml each) were drawn at regular intervals into EDTA-coated tubes containing aprotinin (1,000 KIU/ml blood) for plasma CCK, PYY, and ghrelin determinations.

Part II: effect of free fatty acid hormone release. The design of the second series was similar to part I. The study was conducted in a randomized, double-blind, and three-period crossover fashion; 12 healthy male subjects participated in this part. One treatment consisted of intraduodenal infusion of MCF. MCF in the form of sodium caprylate, a fatty acid with eight carbons, was infused at a concentration of 0.049 g/ml at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h sodium caprylate; this load is equivalent to 26 kcal/h. In the second experiment, LCF in the form of sodium oleate, a LCF with 18 carbons, was infused at a concentration of 0.086 g/ml at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h; this load is equivalent to 46 kcal/h. On the third experimental day, volunteers received intraduodenal vehicle (control) instead of free fatty acids. The MCF and LCF loads were chosen from previous experiments, in which we documented that intraduodenal sodium oleate at a rate of 8 mmol/h induces maximal gallbladder contraction in healthy human subjects (29). During the experiments, 7.5-ml samples of blood were drawn into EDTA-coated tubes containing aprotinin (1,000 KIU/ml blood) for plasma CCK, PYY, and ghrelin determinations.

Part III: effect of LCF with and without intravenous dextroloxi- gumide on ghrelin and PYY secretions. The procedures in this series were similar to part II except for the intravenous and intraduodenal infusions (MCF were not infused in this part). Twelve healthy male subjects participated in this randomized double-blind study. Subjects received on two experimental days a continuous intraduodenal infusion of LCF (sodium oleate, 8 mmol/h) together with either an intravenous infusion of isotonic saline (control) or an infusion of the CCK-1 receptor antagonist dextroloxigiumide (Dexlox; 5 mg kg⁻¹ h⁻¹) for the duration of the study. The dose of Dexlox was chosen from previous experiments (32). Infusions were started 30 min before the intraduodenal infusion. On the third experimental day, subjects received intraduodenal vehicle and intravenous saline during the study. Ambulatory infusion pumps through a Teflon catheter inserted into a forearm vein delivered infusions. Blood was taken (7.5-ml samples) at regular intervals for hormone determinations.

Drugs

Orlistat, also known as tetrahydrolipstatin (Xenical), was purchased from Roche (Basel, Switzerland). Orlistat is poorly soluble in water; 120 mg of orlistat was therefore dissolved in a solution containing 140 ml of distilled water with addition of 4 ml lecithin-ethanol solution plus 1.8 ml saccharose solution. The lecithin-ethanol stock solution was made of 50 ml of 96% ethanol plus 0.75 g lecithin. The saccharose stock solution was prepared by adding 5 g of saccharose to 10.0 ml of distilled water; the mixture was gently vortexed until the saccharose was completely dissolved. An identical solution without orlistat was used as a control solution.

The MCF solution was prepared as follows: 4.9 g of sodium caprylate were dissolved in distilled water by gently stirring for 2 h. After addition of 1.2 ml of 1 M hydrochloric acid, the solution was backtitrated to pH 7.2 with the addition of 1.2 ml of 1 M NaOH. The control solution was identical without addition of sodium caprylate.

Finally, the following procedure was used to prepare the LCF solution: 8.6 g sodium oleate were dissolved in 100 ml distilled water by gently stirring for 2 h. The solution was stabilized at pH 9.5 by addition of 1 M hydrochloric acid to titrate the pH to 9.5. The control solution did not contain sodium oleate. All solutions were infused to the small intestine at a rate of 30 ml/h.

Dexlox, the (R)-isomer of loxiglumide, is a selective and highly potent CCK-1 receptor antagonist; the compound was a kind gift from Dr. Lucio Rovati (Rotta Pharma, Monza, Italy).

Plasma Hormone Determinations

Plasma CCK concentrations were measured by a sensitiveRIA based on an antiserum that recognizes the sulfated tyrosine residue of all CCK molecules but has little cross-reactivity with sulfated gastrin (<1%) and does not cross-react with unrelated gastrointestinal peptides. Plasma samples were extracted with ethanol. 125I-labeled CCK-8 was used as a label. The lowest concentration that could be measured by the assay was 0.6 pmol/l plasma using CCK-8 as a standard. Details of the assay have been previously described (18, 19).

PYY was measured with a commercially available kit (Linco Research, St. Charles, MO). In circulation, PYY exists in at least two molecular forms: PYY(1-36) and PYY(3-36). The antibody, raised in guinea pigs, displays 100% cross-reactivity with human PYY(1-36) and human PYY(3-36) but has no cross-reactivity with human pancreatic polypeptide, neuropeptide Y, and unrelated peptides such as leptin and ghrelin. 125I-labeled PYY was used as a label; the labeled peptide was purified by HPLC (specific activity of 302 μCi/μg). The lowest level of PYY that could be detected by this assay was 10 pg/ml when using a 100-μl plasma sample.

Ghrelin was measured with a commercially available kit (Linco Research). Ghrelin is an octanoylated 29-amino acid peptide. The N-octanoyl group is required for biological activity. The assay measures both octanoylated and des-octanoylated human ghrelin. The lowest level of ghrelin that can be detected by this assay is 93 pg/ml when using a 100-μl sample. At 1 ng/ml, the intra-assay coefficient of variation was 10.0%, whereas the interassay coefficient of variation was 14.7%.

Statistical Analysis

The data were analyzed with nonparametric methods: hormone area under the curve parameters were compared among treatment groups by the Friedman test. If this test revealed statistically significant differences, pairwise comparisons were performed using two-sided Wilcoxon’s signed-rank test, corrected for multiple comparisons by the Bonferroni method. The level of significance was at $P = 0.05$. All statistical comparisons were performed with SPSS for Windows software (version 14.0).

RESULTS

Part I

Ghrelin secretion was significantly inhibited ($P < 0.01$) after intraduodenal fat administration compared with intraduodenal...
In contrast, plasma CCK and PYY concentrations were significantly increased with fat infusion \((P < 0.006\) and \(P < 0.004\), respectively) compared with the control treatment (vehicle). Orlistat (120 mg, infused together with fat) reversed the effects of intraduodenal fat. The inhibition of ghrelin secretion and the stimulation of CCK and PYY release were virtually blocked by the lipase inhibitor; the levels of the three hormones were similar to the control.

**Part II**

Fasting hormone concentrations were comparable in the different treatments. During vehicle infusion (control treatment) and infusion of MCF, plasma ghrelin and CCK and PYY levels remained stable (Fig. 3). Intraduodenal administration of LCF caused a significant inhibition \((P < 0.02)\) of plasma ghrelin concentrations and a significant increase in both plasma CCK and PYY levels \((P < 0.004\) and \(P < 0.008\), respectively) compared with controls (Figs. 3 and 4).

**Part III**

A significant reduction \((P < 0.01)\) in plasma ghrelin concentrations was seen after intraduodenal LCF with intravenous saline compared with intraduodenal vehicle plus intravenous saline (Figs. 5 and 6), similar to the results obtained in part II. Intravenous Dexlox in combination with intraduodenal LCF reversed the inhibition induced by LCF on plasma ghrelin concentrations; the plasma ghrelin levels with Dexlox plus intraduodenal LCF were similar to those in the control treatment; the results were, however, significantly different \((P < 0.02)\) from administration of intraduodenal LCF with intravenous saline (Figs. 5 and 6).
Similar to part II, intraduodenal infusion of LCF with intravenous infusion of saline caused a significant increase (P < 0.004) in plasma CCK compared with controls (intraduodenal vehicle plus intravenous saline). Intraduodenal LCF infusion together with intravenous Dexlox resulted in a significantly augmented CCK response (Figs. 5 and 6) compared with intraduodenal LCF plus intravenous saline (P < 0.004).

Finally, intraduodenal infusion of LCF with intravenous infusion of saline caused a significant increase (P < 0.006) in plasma PYY compared with controls. In contrast, intraduodenal LCF infusion together with intravenous Dexlox resulted in a blunted PYY response compared with intraduodenal LCF plus intravenous saline (Figs. 5 and 6).

DISCUSSION

Ghrelin and PYY are two signals from the gastrointestinal tract that can modulate food intake and appetite (35). PYY inhibits food intake, whereas ghrelin, a peptide synthesized in the stomach, stimulates hunger and increases food intake (4, 5, 9). Further understanding of the physiological mechanisms by which these hormones are regulated by nutrients is required to appreciate the regulatory circuits controlling appetite and food intake.
intake. The different macronutrients exert specific effects on satiety signals. Fat, as an example, is one macronutrient, which has been shown previously to stimulate PYY secretion and to inhibit ghrelin release (14, 15). In the present study, we wanted to extend these observations by analyzing the role of fat digestion in initiating these effects. CCK served as a control parameter in this study, as the effect of fat hydrolysis on CCK release has previously been documented (12, 13, 30).

Three different approaches were used to establish that fat hydrolysis is a critical step in this regulatory circuit. First, we used the lipase inhibitor orlistat as a tool to determine whether inhibition of fat hydrolysis affects the release patterns of ghrelin and PYY. Orlistat is known to inhibit fat hydrolysis from a meal (or in the present study, the duodenal fat infusion) with subsequent effects on CCK release and exocrine pancreatic function (30). Inhibition of ghrelin secretion and stimulation of PYY release in response to intraduodenal fat in the small intestine were completely abolished by orlistat administration. We did not include a formal control treatment (i.e., intraduodenal infusion of orlistat in vehicle alone) in the study, as we have seen in previous investigations that orlistat alone does not have any effect on the digestive system, including hormone release (21). The data are in line with recent studies from the group of Feinle-Bisset (13–15) and our previous findings related to CCK release: suppression of fat hydrolysis by orlistat inhibits the release of CCK (13, 30) and PYY (14, 15). The crucial importance of fat hydrolysis on digestive functions is best illustrated by the effects of these products on exocrine pancreatic secretory responses in animals and humans: only duodenal infusion of long-chain free fatty acids can stimulate maximal pancreatic enzyme and bicarbonate secretion, whereas undigested long-chain triglycerides are ineffective (1, 16, 24, 27, 37). Inhibition of lipolysis by orlistat reduces the amount of free fatty acids in the small intestine.
with a subsequent reduction in CCK release (30). The reduction in CCK release prevents an adequate gallbladder contraction, resulting in a reduced postprandial exocrine pancreatic secretory response, and induces an attenuated inhibitory effect on further food intake (13, 30). The present observations extend the list of digestive functions, which are modulated by intraluminal long-chain free fatty acids. The results imply that the generation of long-chain free fatty acids is a crucial step in the digestive process.

In a second step, we analyzed the importance of chain length of fatty acids in triggering the effects on PYY and ghrelin secretion, respectively. In this part of the study, MCF in the form of sodium caprylate containing eight carbons (denominated MCF) or LCF in the form of sodium oleate with 18 carbons (denominated LCF) was infused into the small intestine. LCF infusion resulted in a significant suppression in ghrelin concentrations and a marked increase in PYY and CCK concentrations. MCF infusion was ineffective: there was no effect on any of the three hormones. These observations suggest that, in addition to fat hydrolysis, the chain length of free fatty acids is crucial for initiating an effect on these two regulatory hormones.

In part III, we used the specific CCK-1 receptor antagonist Dexlox to block the actions of CCK: the suppression of

Fig. 4. AUC for CCK, ghrelin, and PYY during intraduodenal infusions of LCF (8 mmol/h sodium oleate), MCF (8 mmol/h sodium caprylate), or saline (control treatment) in 12 healthy male subjects. Data are means ± SE. LCF infused to the duodenum induced a significant increase in CCK and PYY ($P < 0.004$ and $P < 0.008$, respectively) and a significant suppression of ghrelin concentrations ($P < 0.02$).
LCF-induced ghrelin secretion was reversed and the LCF-stimulated PYY response was abolished with CCK-1 receptor blockade, suggesting that both effects are mediated by CCK via its CCK-1 receptor. Here, we extend previous observations by documenting that adequate fat hydrolysis is required for suppressing ghrelin secretion and for stimulating PYY release. The products of fat digestion stimulate CCK release, which in turn regulates ghrelin and PYY secretions via CCK-1 receptors. It is interesting to compare the plasma profiles of CCK on one hand and ghrelin and PYY secretion patterns on the other hand: the time courses of inhibition of ghrelin and stimulation of PYY make it unlikely that the effects are exclusively mediated by circulating CCK. PYY secretion is initiated either directly via luminal contact of nutrients with the endocrine cells and/or indirectly through neurohumoral signals such as CCK (1, 2, 28). It is conceivable that direct activation of CCK-1 receptors on afferent vagal fibers through LCF stimulation is a potential pathway (3, 7, 8, 23, 25). One type of afferent fiber is sensitive to LCF; furthermore, inhibition of food intake induced by intraduodenal sodium oleate is reversed by bilateral, subdiaphragmatic vagotomy or pretreatment with capsaicin (3, 7, 8, 23, 28, 33). The present observations (CCK-induced suppression of ghrelin and stimulation of PYY) are likely to represent a mechanism through which CCK plays a role in regulating food intake and appetite with fat hydrolysis and the generation of free fatty acids as triggers.

It has been known previously that meal ingestion inhibits ghrelin (11); the effect can be induced by both fat and carbohydrate but not by protein (12–15). Whether carbohydrate-induced inhibition of ghrelin is mediated by CCK through its CCK-1 receptor is not known. PYY secretion is stimulated during meals by the presence of food in the intestine (1, 2, 14,
15, 28); fat in the intestine is the most potent secretagogue for PYY release, whereas carbohydrate and protein are less potent (1). It has not been investigated whether CCK mediates the effects of carbohydrate and protein on PYY secretion.

The plasma CCK levels were markedly higher with Dexlox than with the control treatment. This phenomenon has been observed before with CCK-1 receptor antagonists (6, 20, 26): the explanations include activation of a negative feedback mechanism due to low concentrations of bile and/or pancreatic juice constituents in the duodenal lumen and interference with autoregulating, inhibitory CCK-1 receptors on the I cell.

In summary, the three major issues that relate to the present study are 1) the importance of adequate fat digestion on plasma CCK, ghrelin, and PYY secretions; 2) the role of fatty acid chain length in initiating the effects on ghrelin and PYY secretions; and 3) the consequences of CCK-1 receptor blockade on ghrelin and PYY release. The data support the following concept: fat hydrolysis in the proximal small intestine plays a crucial regulatory function for digestive processes; adequate fat hydrolysis is required to start the process; the specific products of fat digestion, LCF, then stimulate the release of CCK; CCK in turn acts on CCK-1 receptors, which then
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initiate a series of digestive functions, including modulation of ghrelin and PYY secretions. These observations imply that hydrolyzed dietary triglycerides are important for initiating a variety of digestive processes: 1) stimulation of gallbladder contraction and pancreatic enzyme secretion; 2) inhibition of gastric emptying, food intake, and appetite; and finally, 3) regulation of gastrointestinal hormone secretion (inhibition of ghrelin secretion and stimulation of PYY release).

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GRANTS

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