Interaction between GLP-1 and CCK-33 in inhibiting food intake and appetite in men

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Gutzwiller, Jean-Pierre, Lukas Degen, Daniel Matzinger, Sven Prestin, and Christoph Beglinger. Interaction between GLP-1 and CCK-33 in inhibiting food intake and appetite in men. Am J Physiol Regul Integr Comp Physiol 287: R562–R567, 2004. First published April 22, 2004; 10.1152/ajpregu.00599.2003.—Glucagon-like peptide-1 (GLP-1) and CCK-33 were intravenously infused alone or in combination into normal weight men for 60 min before they were served a lunch of ham sandwiches, chocolate mousse, and orange juice. Infusion of GLP-1 (dose: 0.9 pmol·kg−1·min−1) or CCK-33 (dose: 0.2 pmol·kg−1·min−1) each reduced calorie intake of the test meal. However, simultaneous infusion of these peptide doses reduced calorie intake less than the sum of the peptides’ individual effects. Infusions of the same doses of GLP-1 plus CCK-33 had neither individual nor interactive effects on meal size or calorie consumption. The combination of GLP-1 plus CCK-33 induced, however, a significant reduction in hunger feelings in the premeal period (P = 0.036 vs. all other treatments). In summary, intravenous infusion of near physiological doses of CCK-33 and GLP-1 produced specific inhibitions of hunger feeling in men; the simultaneous infusion resulted in an infra-additive reduction in calorie consumption, rejecting thereby the hypothesis that the two peptides exert a positive synergistic effect on food intake compared with the effects observed with infusion of individual peptides. In conclusion, CCK and GLP-1 are meal-related satiety signals that are released from the gastrointestinal tract during food intake.

glucagon-like peptide; cholecystokinin

A SERIES OF REMARKABLE DISCOVERIES and the emergence of obesity as major health problem have stimulated research efforts into how the body controls appetite and food intake. The close relationship between the gastrointestinal endocrine system and the brain in regulating food intake and satiety requires a coordinated interplay in which circulating hormones convey information about food intake and appetite to brain pathways that control eating. However, little is known about which physiological signals interact to control human eating. To further explore the role of specific digestive signals, we investigated the potential interaction of two preabsorptive satiety signals, CCK and glucagon-like peptide-1 (GLP-1). Both peptides are two classical gastrointestinal hormones that are released into the circulation in response to meal consumption (14, 17); compelling evidence has accumulated in the past years to document that each participates in the control of appetite in healthy volunteers, but also in patients with obesity or diabetes type II (3, 8, 11, 15, 25–27). As mentioned before, the control of human eating habits is, however, highly complex and our understanding of appetite regulation is far from complete. In many areas our knowledge is only rudimentary. More important, the interactions between individual signals and their integration into the control system have hardly been explored.

From studies in animals and humans it is known that individual satiety signals can interact: contributions of glucagon and CCK produced functionally synergistic inhibitions of feeding in rats (13, 16), that is, simultaneous injection of the two peptides inhibited feeding significantly more than the sum of their individual effects. In contrast, Geary and coworkers (7) were unable to show in healthy volunteers any interaction between glucagon and CCK; the simultaneous infusion of CCK-8 and glucagon resulted in an infra-additive reduction in meal size, which led the authors to suggest that the two peptides could even interact antagonistically.

At the time of the study, the effects of GLP-1 on food intake and appetite were unknown. From the current perspective, GLP-1 seems to be a better candidate than glucagon in regulating food intake. In addition to its effect on insulin and glucagon secretion, the peptide has important effects on satiety (10, 11). To further explore potential interactions between two well-known satiety signals, we investigated the effects of CCK-33 and GLP-1 and their interaction in the control of food intake and satiety in healthy subjects. We report here that these physiological doses of CCK-33 and GLP-1 each reduced calorie consumption, but that simultaneous CCK-33/GLP-1 infusions produced infra-additive effects on meal size and calorie intake. In contrast, intravenous infusion of GLP-1 and CCK-33 had little effect on feelings of hunger, but simultaneous GLP-1/CCK-33 infusions produced a synergetic effect on hunger feelings in the premeal period.

METHODS

Subjects. Healthy male subjects were screened as previously described (8, 11). Inclusion criteria were 1) body mass index (BMI) within 15% of normal; 2) age between 20 and 35 yr; 3) nonsmoker; 4) history of good health, no active medical problems, and taking no medication; 5) no history of food allergy or dietary restriction; and 6) normal physical examination and laboratory screening.

Participants were given a written description of the study and written informed consent was obtained from each participant. The protocol was approved by the local Ethical Committee for Human Studies. Twenty-four paid male volunteers completed the study (mean age 23 yr, range 21–29 yr, BMI 23.2 ± 0.8).

Overall design. The study employed a randomized, placebo-controlled, double-blind, four-way crossover design in which each subject underwent four tests: either saline, CCK-33, GLP-1, or CCK-33 plus GLP-1 was infused. Test trials were separated by at least 7 days.

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Experimental procedure. Four treatments, separated by at least 7 days, were performed in each subject. On the day of each experiment, subjects ate a small breakfast, but no snacks were allowed after 8:00 AM. At noon, the experiment started with a continuous infusion. The treatments were identical in design except for the intravenous infusion (see Fig. 1).

The first treatment consisted of an intravenous GLP-1 infusion (dose: 0.9 pmol·kg⁻¹·min⁻¹) for the duration of the experiment. This dose was chosen from previous experiments (8, 12). Sixty minutes after starting the infusion, subjects were invited to eat and drink as much as they wished. The second treatment was similar. Intravenous CCK-33 (dose: 0.2 pmol·kg⁻¹·min⁻¹) was given during the whole experiment. The third experiment used intravenous CCK-33 plus GLP-1 throughout the entire experiment. The fourth experiment used intravenous saline throughout the entire experiment instead of hormones (control study).

Sixty minutes after starting the infusions, a standard meal (Table 1) was presented to the subjects and they were invited to eat and drink as much as they wished for 60 min. The meal consisted of 1) orange juice; 2) ham sandwiches (60 g wheat bread, 10 g butter, and 25 g ham); 3) chocolate pudding; and 4) coffee with cream and sugar (coffee could be sweetened if desired; therefore both cream and sugar were optional). The order of food intake had to follow the above schedule. To reduce the participant’s awareness of the amount of food eaten, food was presented in small samples and in excess.

The intravenous infusions were indistinguishable in appearance from the control solution (saline) and the person in charge of the infusions was unaware of the respective treatment, thereby making it possible to conduct treatments in a double-blind fashion. The amount of food eaten, the volume of fluid drunk, and the time for each subject to complete the meal were quantified. From these observations, total caloric intake could be calculated. Before and during the premeal period, blood was drawn for glucose and plasma CCK and GLP-1 determinations. Blood was drawn in indwelling antecubital catheters into syringes on ice. These contained EDTA (6 μmol/l) and aprotinin (1,000 kIU/l). After configuration, plasma samples were kept frozen at −20°C until analyses. Plasma glucose was analyzed by the glucose-oxidase method.

After the start of the perfusion, subjects scored their subjective feelings for hunger and fullness at 15-min intervals for the duration of each experiment using a visual analog scale from 1 through 10 and indicated their scores on a questionnaire. The scale and scores were previously designed and described by Welch and coworkers (28, 29). In brief, a score of 0 for hunger indicated that the subject was not hungry at all, 2 indicated “slightly hungry,” 5 indicated “moderately hungry,” 8 indicated “very hungry,” and 10 indicated “absolutely ravenous.” The score for fullness was similar.

Peptides. For the peptide infusions, commercially available synthetic human GLP-1(7–36)amide and synthetic human CCK-33 were purchased from Bachem, Bubendorf, Switzerland. The peptides were dissolved in 0.9% saline solution containing 0.5% human serum albumin. The peptides were prepared under aseptic conditions by the University of Basel Hospital Pharmacy. Aliquots of 50 μg/5 ml were stored at −20°C. Infusion solutions were prepared by diluting appropriate amounts of GLP-1 with 0.9% saline containing 0.1% human serum albumin. Control solutions contained 0.1% human serum albumin alone; they were indistinguishable in appearance from peptide infusions. The solutions were prepared by a person who was not involved in the study. The physician in charge of the experiment was therefore not aware of the respective treatment, thereby making it possible to conduct treatments in a double-blind fashion.

Biochemical analyses. Plasma CCK was determined by radioimmunoassay previously published (12). The antibody is directed to the sulfated tyrosyl region with negligible cross-reactivity with sulfated forms of gastrin (<1%); the antibody does not bind to unsulfated forms of gastrin or structurally unrelated peptides (details are given in Table 2). The detection limit of the assay was 0.6 pmol/l, and intra-assay and interassay precisions were <13%, respectively. Before radioimmunoassay, plasma samples were extracted with ethanol (final concentration: 70% vol/vol) (12). GLP-1 immunoreactivity was measured as described previously (10, 11). The antisera is specific for GLP-1 and does not cross react with any other members of the glucagon family of peptides. The detection limit of plasma was 0.25 pmol/l. The intra-assay and interassay coefficient of variation were <8% and <14%, respectively. Before radioimmunoassay, plasma samples were extracted with ethanol (final concentration: 70% vol/vol).

Blood glucose concentrations were measured by a commercial hexokinase-glucose-6-phosphate-dihydrogenase method (Roche, Basel, Switzerland).

Statistical analysis. The amount of food eaten (g) and the amount of fluid drunk (ml) including their corresponding energy intake (kcal) were compared between the treatments by ANOVA. In the event of significant differences, ANOVA was followed by multiple paired t-tests with Bonferroni’s correction. The same statistical procedure was used to analyze the results of plasma glucose, plasma GLP-1, and plasma CCK concentrations using area under the curve analysis. The scores for hunger and fullness were compared at the different time points before and after meals, with repeated-measures ANOVA.

To determine whether CCK-33 and GLP-1 inhibited food intake synergistically, the amount of food eaten and the respective caloric intake were analyzed with factorial ANOVA (factors were CCK-33 and GLP-1) followed by planned comparisons with Tukey’s honestly significant difference test. Positive synergism was postulated to occur if the combined infusion effect was significantly larger than the sum of the individual effects.

Table 1. Composition of test meal with corresponding nutritive values

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Carbohydrates (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice (100 ml)</td>
<td>10</td>
<td>0.7</td>
<td>n/a</td>
<td>44</td>
</tr>
<tr>
<td>Ham sandwich (100 g)</td>
<td>35.8</td>
<td>12.3</td>
<td>12.5</td>
<td>305</td>
</tr>
<tr>
<td>Chocolate pudding (100 g)</td>
<td>19.2</td>
<td>3.6</td>
<td>7.2</td>
<td>172</td>
</tr>
</tbody>
</table>

Energy conversion relied on manufacturer’s specifications.

Table 2. Characteristics of CCK antibody

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Cross Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK-8, sulfate</td>
<td>100%</td>
</tr>
<tr>
<td>CCK-33, sulfate</td>
<td>134%</td>
</tr>
<tr>
<td>CCK-8, nonsulfated</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Gastrin-17, sulfate</td>
<td>0.5%</td>
</tr>
<tr>
<td>Gastrin-17, nonsulfated</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>CCK-4</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>
To determine the effect of CCK-33, GLP-1, and the combined treatment on hunger feelings, the difference in hunger feelings during the premeal period was obtained subtracting hunger feelings at 60 min from the baseline value. The differences between the treatments were compared using the techniques described above.

RESULTS

Food intake. CCK-33 given alone decreased the amount of food eaten (nonsignificant) with a consecutive reduction in calorie intake (P = 0.0006) compared with saline infusion (Table 3). Fluid consumption was not significantly decreased by CCK-33. GLP-1 given alone did not significantly alter the amount of food eaten; the calorie consumption was, however, significantly reduced (P = 0.0036).

A positive synergistic effect between CCK-33 and GLP-1 was not detected. Simultaneous infusions of the two peptides produced similar effects on the amount of food eaten and on calorie consumption as was achieved with infusion of individual peptides (Table 3). Therefore no additive interaction was seen. To the contrary, the sum of their individual effects is greater if the food inhibitory effects of CCK-33 and GLP-1 are added compared with the effects of combined intravenous infusion.

None of the participants reported any abdominal discomfort or side effects during infusion of CCK-33, GLP-1, or the combination of CCK-33 plus GLP-1. Furthermore, when questioned at the end of each experiment, none of them experienced or reported any adverse reaction.

Subjective ratings of hunger. Reports of subjective experiences during control tests are given in Fig. 2. CCK-33 and GLP-1 induced a nonsignificant reduction in hunger feelings when baseline scores were compared with the 60-min values. The most potent effect was seen with the combination of concomitant CCK-33 plus GLP-1 infusion: subjects felt markedly less hungry in the premeal period compared with saline administration. When we compared differences of hunger score between 60 min and baseline score values, the difference reached statistical significance (P = 0.036 vs. saline). Ingestion of the test meals rapidly decreased ratings of hunger scores.

Subjective ratings of fullness. Reports of subjective experiences during control tests and peptide infusions were similar and did not reveal statistically significant differences. Ingestion of the test meals rapidly increased ratings of fullness scores (data not shown).

Blood glucose concentrations. With GLP-1 infusions, blood glucose levels decreased (Fig. 3); this effect provides experimental evidence on the biological activity of GLP-1.

Table 3. Inhibitory effects of GLP-1, CCK-33 or a combination of GLP-1 plus CCK-33 on food intake in healthy male subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CCK-33</th>
<th>GLP-1</th>
<th>CCK-33 plus GLP-1</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food quantity, g</td>
<td>565±25</td>
<td>610±29</td>
<td>636±24</td>
<td>613±25</td>
</tr>
<tr>
<td>Calorie intake, kcal</td>
<td>1,561±38*</td>
<td>1,605±49†</td>
<td>1,630±50‡</td>
<td>1,760±35</td>
</tr>
<tr>
<td>Fluid intake, ml</td>
<td>799±37</td>
<td>697±32</td>
<td>711±39</td>
<td>733±29</td>
</tr>
</tbody>
</table>

Data are means ± SE, n = 24. *CCK-33 vs. saline (P = 0.0006). †GLP-1 vs. saline (P = 0.0036). ‡CCK-33 plus GLP-1 vs. saline (P = 0.027).

DISCUSSION

The investigation of human eating behavior, especially the regulation of appetite and satiety, has become a very active field of research with the potential for the development of a specific therapy for obesity. We still have, however, limited information about the biochemical processes that control hunger and satiety. In the past years, important information has been obtained that helps to elucidate the control circuits in these processes. A series of recent studies in humans has...
established that both peripherally administered CCK and GLP-1 can reduce energy intake and modulate subjective appetite sensations (6, 10, 14, 19, 20, 27). The present study was designed to explore potential interactions between these two preabsorptive satiety signals.

The major findings of this study can be summarized as follows. First, the intravenous infusion of physiological doses of GLP-1 or CCK-33 reduced calorie intake in healthy, male subjects. Second, a similar inhibition was seen when the same doses of GLP-1 and CCK-33 were infused together. These data are consistent with the hypothesis that both GLP-1 and CCK can accelerate postprandial satiety and that both peptides are endocrine satiety signals. However, no additive interaction was observed between the two peptides with respect to calorie intake. Third, both GLP-1 and CCK-33 had small, but nonsignificant effects on hunger feelings in the premeal period. A marked inhibition of hunger occurred, however, when these same GLP-1 and CCK-33 doses were infused together.

GLP-1 and CCK have previously been reported to reduce food intake and appetite in humans (6, 19, 20); the present results with individual hormone application confirm these previous results. A recent meta-analysis of the effect of GLP-1 infusion demonstrated an average reduction in calorie intake of 11.7% (27); with GLP-1 infusion alone, we saw a comparable 8.8% reduction in calorie consumption. In previous studies with CCK given intravenously at physiological levels (19, 20) to normal weight human volunteers, the amount of food eaten was reduced by 12% compared with 9% in the present study; calorie intake was not quantified in this previous report (here we observed an 11.3% reduction in calorie intake). Here we extend these observations and tested whether simultaneous administration of GLP-1 and CCK-33 caused additive inhibitory effects. A previous study in rats documented that simultaneous administration of glucagon and CCK-33 caused more than additive effects because the combined administration inhibited feeding more than the sum of the individual effects of the peptides (13, 16). In a follow-up study in healthy male subjects, the simultaneous infusion of CCK-33 plus glucagon resulted in an infra-additive reduction in meal size, which led the authors to propose that the two peptides could interact antagonistically (7). The time courses of postprandial CCK and glucagon concentrations are, however, not parallel; combined infusion of glucagon and CCK does not therefore completely reflect normal physiology. Both CCK and GLP-1 are, on the other hand, markedly stimulated by meal ingestion and their time courses are quite similar. Combined infusion of CCK and GLP-1 mirrors therefore within the limits of the present model the normal postprandial situation. The results of this study reveal, however, that no positive interaction was present with respect to food or calorie intake. Combined infusion of these peptides reduced food consumption and calorie intake significantly less than the sum of their individual effects. We conclude that CCK and GLP-1 do not interact in the regulation of food consumption.

How can these results be explained? Both CCK and GLP-1 exert inhibitory effects on gastric emptying rates and these effects may at least in part be responsible for their satiety-producing properties (18, 23). The interaction of CCK and GLP-1 on gastric emptying has not been investigated so far; it is therefore unknown whether combined infusion of CCK plus GLP-1 exerts additive effects on gastric emptying or whether the effect of the combined infusion is similar to the effect induced by a single infusion of one of the two peptides. However, it is known that inhibition of gastric emptying and inhibition of food intake can be independent actions. The results of the visual analog scales are instructive for this discussion. The hunger scores were significantly lower during the combined CCK-33 plus GLP-1 infusions compared with all other treatments. We infer from these observations that CCK-33 and GLP-1 interact to reduce hunger feelings. Suppression of plasma ghrelin concentrations could be a potential explanation for this observation. An inverse relationship between circulating levels of ghrelin and GLP-1 has been recently described (4), which may indicate an interaction regarding regulation of the secretion of the two peptides. Unfortunately, we did not measure ghrelin concentrations in the present study. Also, it is important to stress that the presence and the relevance of this ghrelin/GLP-1 interaction needs to be confirmed, preferably by interaction studies in which healthy subjects are exposed to ghrelin and GLP-1 or both.

Several potential limitations of our study should be recognized. First, only one dose of each peptide was studied; the doses were chosen from our previous studies and aimed to match physiological plasma concentrations of both CCK and GLP-1. Second, only male subjects were investigated as pre-

Fig. 4. Plasma CCK concentrations (A) and GLP-1 concentrations (B) during the 4 treatments in 24 healthy male subjects during the premeal period. Data are means ± SE.
vious findings suggest that young male volunteers have the highest capacity to regulate energy intake in response to energy manipulation (21, 22). As a consequence, our observations are valid for young male subjects but cannot necessarily be extrapolated to female subjects.

The mechanism(s) by which CCK and GLP-1 modulate food intake in humans is not clear. Presumably, inhibition of gastric emptying may in itself cause a limitation of food intake through either neural or endocrine signaling pathways, perhaps associated with gastric distension (5). Both CCK and GLP-1 may, however, interact with other gastrointestinal signals, which may be stimulated by food intake: gastrin-releasing peptide and peptide YY (PYY) (1, 9, 24). The precise role of individual peptides can, however, only be determined when a specific and sufficiently potent antagonist becomes available for human use.

Many questions remain unanswered. Does CCK or GLP-1 interact with other putative gastrointestinal satiety hormones released in response to meal intake (e.g., PYY)? PYY-(3–36) would seem to be a very promising candidate for such an interaction: 1) the peptide is colocalized with GLP-1 in L-cells of the distal small intestine; they are released synchronously after food intake from the distal small intestine in proportion to the calorie content of the meal (1, 2); 2) they exert additive inhibitory effects on gastric acid secretion (1); 3) CCK stimulates the release of PYY-(3–36) (1); 4) PYY-(3–36) inhibits food intake in normal mice and rats but not in Y2-receptor negative mice (2); in humans, infusion of normal postprandial concentrations of PYY-(3–36) significantly decreases appetite and reduces food intake by 33% over 24 h (1, 2). Thus postprandial elevation of PYY-(3–36) seems to be one of the most potent satiety signals in man. Further research is required to determine whether PYY-(3–36) interacts with other satiety factors of the gastrointestinal tract.

In summary, intravenous infusion of near physiological doses of CCK-33 and GLP-1 produced specific inhibitions of feeling in men; the simultaneous infusion resulted in an infra-additive reduction in calorie consumption, rejecting thereby the hypothesis that the two peptides exert a positive synergistic effect on food intake compared with the effects observed with infusion of individual peptides. The combined infusion of the peptides induced on the other hand a marked reduction in hunger feelings in the premeal period. These data are consistent with the hypothesis that both CCK and GLP-1 can accelerate satiety independent of their effects on gastric emptying. In conclusion, CCK and GLP-1 are meal-related satiety signals that are released from the gastrointestinal tract during food intake. Both peptides promote a sense of fullness that encourages an end to the meal. Therefore both peptides are factors that trigger the termination of eating, participating in a meal-to-meal control system. Much more information is necessary to understand the basic physiological mechanisms that control food intake and satiety. Elucidation of these mechanisms will increase the understanding of the role of specific-satiety inducing control circuits in the regulation of appetite and body weight and the development of overweight and obesity.

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GRANTS

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