Interaction between CCK and a preload on reduction of food intake is mediated by CCK-A receptors in humans

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Gutzwiller, Jean-Pierre, Juergen Drewé, Silvia Ketterer, Pius Hildebrand, Alexandra Krautheim, and Christoph Beglinger. Interaction between CCK and a preload on reduction of food intake is mediated by CCK-A receptors in humans. Am J Physiol Regulatory Integrative Comp Physiol 279: R189–R195, 2000.—Cholecystokinin (CCK) interacts with neural signals to induce satiety in several species, but the mechanisms are unclear. We therefore tested the hypothesis that alimentary CCK (CCK-A) receptors mediate the interaction of CCK with an appetizer on food intake in humans. CCK octapeptide (CCK-8, 0.75 μg infused over 10 min) or saline (placebo) with concomitant infusions of saline (placebo) or loxiglumide, a specific CCK-A antagonist, was infused into 16 healthy men with use of a double-blind, four-period design. All subjects received a standard 400-ml appetizer (amounting to 154 kcal) but were free to eat and drink thereafter as much as they wished. The effect of these infusions on feelings of hunger and satiety and on food intake was quantified. CCK-8 induced a reduction in calorie intake (P < 0.05) compared with saline. Furthermore, a decrease in hunger feelings (P < 0.05, saline-CCK-8 vs. all other treatments) and an increase in fullness were observed. These effects were antagonized for hunger and fullness by loxiglumide. We conclude that CCK-8 interacts with an appetizer to modulate satiety in humans. These effects are mediated by CCK-A receptors.

THE EFFECT ON FOOD INTAKE resulting from the interaction between a cholecystokinin (CCK) infusion and gastric distension has previously been explored in humans (26). Different levels of gastric distension were induced by giving variable amounts of a liquid preload to healthy volunteers together with a short-term infusion of intravenous CCK octapeptide (CCK-8) before a regular meal was presented. The results suggested that gastric distension interacts with CCK-8 to reduce food intake in humans. This interaction was first reported in rats (1): a greater reduction in food intake was observed when CCK-8 was injected after the animals had already eaten some food than when CCK-8 was given without a preload of food. However, the rat study used a sham-feeding preparation, which may have obscured the contribution of the gastric phase to the regulation of food intake. A clear role for gastric distension has therefore not been established, and it is possible that an interaction of gastric distension with oral and intestinal stimuli is responsible for the reported effects. However, several additional lines of evidence support the hypothesis that CCK can suppress food intake in a variety of animals and also in humans (2, 6–8, 14, 16, 18, 23, 35, 39).

Further exploration of the interaction between CCK and the stomach thus appeared to be a fruitful line of investigation to better understand the regulation of food intake in humans. The availability of potent and selective alimentary CCK (CCK-A) receptor antagonists has made it possible to continue these investigations. Loxiglumide (Lox) is one of these specific CCK-A receptor antagonists available for human use (11, 12, 32, 33). Lox is therefore a useful tool to test the hypothesis that peripheral CCK-A receptor types are responsible for mediating the interactions of CCK with a preload on food intake in humans.

METHODS

Subjects. Thirty-two men, aged 21–33 (mean 24) yr, participated in the study. The weight of all subjects was within a normal range for their age, gender, and height. Each subject gave written informed consent for the study. The protocol was approved by the Human Ethics Committee of the University Hospital in Basel. Before acceptance, each participant was required to complete a medical interview, received a full physical examination, and participated in an initial laboratory screening. No subject was taking any medication or had a history of food allergies or dietary restrictions.

Experimental procedure. A randomized, double-blind crossover design was used for four treatments, separated by ≥7 days, in each subject. On the day of the experiment, each subject ate breakfast if this was his normal custom, but no snacks were allowed after 8 AM. At noon, the experiment started with the first continuous infusion. The treatments were identical in design, except for the intravenous infusions (Fig. 1). Sixteen subjects participated in the study.

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The first treatment consisted of an intravenous saline infusion for the duration of the experiment and a second saline infusion given for 10 min. The second treatment was similar: intravenous saline was given during the whole experiment, but CCK-8 (0.75 µg infused at a rate of 1 ml/min over 10 min) was administered as a second short infusion instead of saline. The third and fourth treatments used intravenous Lox (10 mg · kg⁻¹ · h⁻¹) throughout the entire experiment instead of saline, combined with either saline or CCK-8, in the dose mentioned above, as short infusions. The dose of Lox was chosen from previous experiments (11, 12, 32, 33). This dose of Lox is able to abolish meal-stimulated gallbladder contraction.

After having started the infusion, the subjects scored their subjective feelings for hunger and fullness at 15-min intervals throughout each treatment by use of a visual analog scale from 1 through 10 and indicated their scores on a questionnaire. The scale and scores were designed and described in detail previously (4, 37, 38). Briefly, 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.

At 45 min after the start of the infusion of Lox or saline, a preload of 400 ml of banana shake was served to the subjects. The preload used in this study is based on experiments performed by Muurahainen et al. (26). The shake was made of 100 g of whey, 16 g of sugar, and 100 g of blenderized banana and mixed with water to a total amount of 400 ml. The composition of the preload was 35.8 g of carbohydrates, 0.4 g of fat, and 1.7 g of protein amounting to 154 kcal. At 15 min after ingestion of the preload, the second infusion of CCK-8 or saline was started and administered for 10 min.

At 5 min after the start of this second infusion, a standard meal 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 (wheat bread, butter, and ham), 3) chocolate pudding, and 4) coffee. Coffee could be sweetened with sugar if desired, and cream could be added (5 g sugar = 20 kcal, 12 g cream = 20 kcal). The order of food intake had to follow the above schedule. The nutritive values of the test meals are given in Table 1. The energy conversion relied on manufacturer’s specifications and was not controlled by calorimetry. To reduce the participant’s awareness of the amount of food eaten, food was presented in small samples and in excess.

Table 1. Composition of the test meal with corresponding nutritive values

<table>
<thead>
<tr>
<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>44</td>
</tr>
<tr>
<td>Ham sandwich, 100 g</td>
<td>35.8</td>
<td>12.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Chocolate pudding, 100 g</td>
<td>19.2</td>
<td>3.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Participants were able to sit, eat, stand, and walk comfortably while receiving the infusions. The CCK-8-Lox solutions were indistinguishable in appearance from the control solution (saline), and the person in charge of the experiments was unaware of the respective treatment, thereby making it possible to deliver 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. Before, during, and after the preload, blood was drawn for plasma CCK determinations in EDTA-coated tubes containing aprotonin (1,000 kallikrein-inactivating units/ml blood).

Plasma hormone determinations. Plasma immunoreactive CCK concentrations were measured by a sensitive RIA based on the antiserum OAL-656, which recognizes the sulfated tyrosine residue of CCK-8 but has no relevant cross-reactivity with sulfated gastrin (<1%) and does not cross-react with unrelated gastrointestinal peptides (10). Plasma samples were extracted with ethanol. The detection limit of the assay was 5 pg/ml plasma with CCK-33 as a standard. Details of the assay have previously been described (12, 20, 21).

Statistical analysis. The amount eaten and drunk and the corresponding calorie intake were analyzed with repeated-measures ANOVA on treatments with use of the GLM procedure in the SAS software package (30). In the event of significant differences, ANOVA was followed by Scheffé’s multicomparison test for pairwise comparisons (29). The scores for hunger and fullness were compared at the different time points before and after meals, with GLM repeated-measures ANOVA accounting for treatment effects as well as between-subject factor. Scheffé’s test was used as a post hoc multicomparison test for treatment effects (29, 30).
RESULTS

Food intake. The results on the different food variables are given in Table 2. They can be summarized as follows. 1) The amount of food consumed was significantly less after saline-CCK-8 ($P < 0.001$) than after all other conditions, which were not different from each other. 2) Fluid consumption was significantly ($P < 0.02$) reduced after saline-CCK-8 compared with saline-saline, but the difference did not reach statistical significance ($P = 0.054$). However, when the two CCK treatments were averaged across the two levels of Lox (i.e., CCK-Lox + CCK-saline), the difference from the saline levels (saline-Lox and saline-saline) was significant, and CCK reduced energy intake by 184 ± 48 kcal ($t = 3.83, P = 0.04, 45$ degrees of freedom). In fact, the only significant differences for total energy intake were between saline-CCK-8 and Lox-CCK-8 and between saline-CCK-8 and Lox-saline (Table 2). Thus CCK-8 alone did not significantly reduce calorie intake, but Lox-saline significantly raised calorie intake compared with saline-saline. Therefore, in the present study, CCK-8 significantly decreased energy intake only when Lox was given. Lox, on the other hand, increased calorie consumption regardless of whether CCK-8 or saline was infused.

To further analyze potential interactions, the following contrasts were calculated: saline-saline – CCK-8-saline – (saline-Lox – CCK-8-Lox). These data are given in Table 3. The calculations provide support for the statements that CCK-8 indeed reduced the amount of food eaten and the amount of fluid consumed and that these effects were reversed by Lox. No significant effect was seen for calorie intake, however.

No overt side effects were observed by the attending physician during the different treatments. None of the volunteers reported sickness or nausea; when they were questioned later, no adverse events were reported.

Eating ratings. As expected, hunger ratings fell and fullness ratings rose after the subjects had drunk the preload. The effect on the visual analog scale was most pronounced with saline-CCK-8 and was statistically significant ($P < 0.05$) during the CCK-8 infusion compared with all other treatments (Fig. 2). In fact, CCK-8 did significantly reduce hunger and increased fullness in the premeal period, and these effects were reversed by Lox. Lox alone, on the other hand, did not significantly affect hunger or fullness feelings. These data indicate that CCK-8 interacts with a preload to induce

Table 2. Effect of Lox or saline and short-term infusions of saline or CCK-8 on feeding behavior in healthy men

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline-Saline</th>
<th>Saline-CCK-8</th>
<th>Lox-Saline</th>
<th>Lox-CCK-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories intake</td>
<td>1,797 ± 68</td>
<td>1,675 ± 83†</td>
<td>1,983 ± 48</td>
<td>1,736 ± 54</td>
</tr>
<tr>
<td>Amount of food, g</td>
<td>645 ± 30</td>
<td>421 ± 35‡</td>
<td>597 ± 21</td>
<td>560 ± 24</td>
</tr>
<tr>
<td>Amount of fluid, ml</td>
<td>760 ± 43</td>
<td>592 ± 36§</td>
<td>706 ± 34</td>
<td>688 ± 33</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 16$. Cholecystokinin octapeptide (CCK-8, 0.75 μg) or saline was infused over 10 min. Lox, loxiglumide. *$P = 0.015$, saline-CCK-8 vs. Lox-saline. †$P = 0.054$, saline-CCK-8 vs. Lox-CCK-8. ‡$P < 0.001$, saline-saline vs. saline-CCK-8 saline; $P = 0.001$, saline-CCK-8 vs. Lox-saline; $P = 0.01$, saline-CCK-8 vs. Lox-CCK-8. §$P = 0.02$, saline-saline vs. saline-CCK-8.

Table 3. Effect of treatments on food parameters in healthy men

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calorie Intake</th>
<th>Amount of Food</th>
<th>Amount of Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>124 ± 96</td>
<td>187 ± 42</td>
<td>148 ± 69</td>
</tr>
<tr>
<td>$t$</td>
<td>1.3</td>
<td>4.44</td>
<td>2.16</td>
</tr>
<tr>
<td>$P$</td>
<td>0.207</td>
<td>0.0001</td>
<td>0.0361</td>
</tr>
</tbody>
</table>

Values are means ± SE. Intraclass differences between treatments were calculated by the formula: saline-saline – saline-CCK-8 – (Lox-saline – Lox-CCK-8).

Fig. 2. Subjective sensations for hunger (A) and fullness (B) experienced by healthy volunteers before and after food intake during intravenous infusion of saline or loxiglumide (Lox, 10 mg·kg⁻¹·h⁻¹) together with short-term infusions of CCK-8 (0.75 μg over 10 min) or saline. At 20 min before food consumption, participants received a preload (400 ml of banana shake). Values are means ± SE ($n = 16$).

*Significant difference between saline-CCK-8 and saline ($P < 0.05$).
early satiety, an effect that is mediated by CCK-A receptors. No statistical differences in hunger or fullness ratings were observed during meal intake for fullness or hunger scores between any treatment.

Plasma CCK. During the control treatment (saline-saline), plasma CCK remained stable in the premeal period. Banana shake (400 ml) did not stimulate plasma CCK concentrations (Fig. 3). During CCK-8 infusion, a significant increase (P < 0.01) in plasma CCK levels was obtained. Interestingly, in the treatment with intravenous Lox administration, the preload of banana shake induced a significant increase in plasma CCK (P < 0.05). The highest increase in plasma CCK was seen with CCK-8-Lox (Fig. 3).

DISCUSSION

The role of the preabsorptive release of CCK in the production of meal-ending satiety has been extensively studied in animals (5, 9, 24, 27, 35). The evidence in humans for CCK as a peripheral satiety signal is less clear. In 1981, Kissileff et al. (14) reported that infusion of exogenous CCK-8 decreased liquid meal intake in human volunteers. Recently, Lieverse and co-workers (18) extended these observations by showing that CCK infusions leading to physiological plasma CCK levels significantly increased satiety in humans. However, not all studies found consistent effects of CCK on satiety in humans. Infusion of exogenous CCK at plasma levels termed physiological (because they induced postprandial gallbladder contraction or pancreatic enzyme secretion) failed to affect food intake and satiety effects in healthy human subjects in at least two studies (19, 31). Different results have also been obtained with different specific CCK-A receptor antagonists in healthy volunteers: oral administration of MK-329, also known as devazepide, induced a significant increase in hunger feelings, suggesting that endogenous CCK is indeed involved in the physiological control of food intake (40). Our previous results with Lox are in contrast with the findings obtained with MK-329: intravenous infusion of the CCK-A receptor antagonist Lox failed to reverse fat- or phenylalanine-induced inhibition of food intake and satiety, suggesting that CCK is not important under these circumstances (4, 28). It is, however, doubtful that these last experiments used the optimal conditions for demonstrating CCK’s effectiveness. The compounds (fat and phenylalanine) were perfused to the duodenum, thereby bypassing the stomach, whereas a large gastric distension accompanied by CCK release seems to be important for CCK to have an effect (4, 25, 26, 28). Also, rather large loads of fat and phenylalanine were used in these last experiments, and this may have affected the results by possibly disturbing gastrointestinal motility and thus overruling the satiety effects of CCK. In support of the role of CCK are additional recent observations of Lieverse and co-workers (17), who demonstrated that a small load of intraduodenal fat increased satiety mainly through the effects of CCK.

With this background in mind, we extended the hypothesis of Muurahainen et al. (26), which suggested that a preload of a liquid meal with a concomitant infusion of CCK-8 can reduce food intake in humans. As another extension to the previous work, the CCK-A receptor antagonist Lox was used as a tool in the present experiments. The results show that a preload of banana shake together with CCK-8 infusion induces a significant reduction in food intake compared with the control experiment (saline-saline infusion). The effects of CCK-8 seemed to be specific, inasmuch as the CCK-A receptor antagonist Lox was able to antagonize the effects of CCK-8. The average calorie intake was even increased by ~10% in the experiments with Lox-saline administration, suggesting indeed that CCK-8 through its A receptor modulates food intake in humans. We interpret the results with Lox as compelling evidence that endogenous CCK released by food ingestion curtails hunger feelings and induces early satiety in humans, implying that CCK is indeed one of several physiological satiety signals in humans.

Fig. 3. Plasma CCK concentrations in the premeal period during short-term infusion of CCK-8 (0.75 μg over 10 min) or saline. Values are means ± SE. * Significant difference between CCK-8 and saline. † Significant difference between saline and Lox.
This study differs from various other human studies in several respects. In previous studies (14, 25, 36) in which CCK-8 induced satiety, CCK plasma levels were not measured, and it is very possible that unphysiological plasma CCK levels were obtained. Here, we used two different approaches: 1) we infused CCK-8 at a low concentration, and 2) we used a specific CCK-A receptor antagonist as a specific tool.

Along these lines, we have shown that CCK indeed can modulate satiety in humans and that these effects are probably mediated by CCK-A receptors. Which mechanism is responsible for the satiety effect of CCK? Observations based mainly on animal work suggest that the inhibitory effects of CCK on gastric emptying rates may contribute to its satiety-producing properties (3). These findings could explain the negative results obtained in the study with Lox and intraduodenal nutrients where the stomach was bypassed (4, 28). However, even though intravenous CCK has been shown to slow gastric emptying of liquid meals in humans (6, 16, 23), it has to be kept in mind that inhibition of gastric emptying and inhibition of food intake can be independent actions. The possibility even exists that CCK primarily acts on satiety through signals that act exclusively on abdominal vagal afferent fibers but are unrelated to gastric distension or gastric emptying rates. Along this line of argumentation, Joyner and co-workers (13) observed in rats that the satiety potency of CCK-8 was equivalent in sham- and real-feeding experiments, thus implying that under certain conditions the satiating effect is independent of gastric distension, because gastric distension does not occur during sham feeding (13). In contrast to this rat study, Melton and co-workers (22) noted in women that ratings of hunger were higher with CCK-8 than with saline administration when a gastric balloon was inflated to the same gastric pressure. In addition, fullness ratings rose and hunger feelings declined more steeply in relation to gastric pressure when CCK-8 was infused. These data indicate that the stomach is important in amplifying signals that regulate hunger and satiety independently of other aspects such as food composition.

When the plasma CCK concentrations are compared with digestive processes such as postprandial gallbladder contraction, they can be termed physiological. However, it is not known what level of CCK is necessary to achieve concentrations at the receptor that are able to reduce food intake. The banana shake itself did not release any CCK. Therefore, it is unlikely that circulating levels of CCK mediated these effects. The lack of increase in CCK is not surprising, because we chose a preload composed mainly of carbohydrates, which are poor CCK secretagogues (15). In agreement with previous observations, we noticed that Lox did not influence basal plasma CCK concentrations (11, 23, 34), but it did induce a considerable increase in plasma CCK after the preload was given. This finding is interesting, inasmuch as it suggests that gastric distension participates in the control of CCK release, but these observations require further confirmation. An augmented CCK release during Lox administration has previously been documented and related to a diminished bile acid and/or enzyme concentration in the lumen of the proximal small intestine because of diminished gallbladder emptying or inhibition of exocrine pancreatic secretion (11, 23, 34). This increase in plasma CCK has been explained by a feedback control of intraluminal bile and/or enzyme concentration, which, in turn, regulates CCK release (11, 23, 34).

The results of the visual analog scales are instructive. The fullness score was significantly higher only during CCK-8 infusions. In parallel, the hunger ratings were lower with saline-CCK-8. Lox completely blocked this effect, demonstrating that it is mediated by peripheral CCK-A receptors. Lox alone induced a tendency for greater hunger feelings and lower fullness signals, implying that these effects were truly mediated by CCK-A receptors.

In conclusion, the results in our study lend support to the hypothesis that CCK-like peptides are endogenous signals involved in the control of food intake in humans; these signals are mediated by CCK-A receptors. Further investigations are needed to define the adequate stimuli for CCK and its role in the control of human food intake. Given the importance of reducing fat and calorie intake for public health to understand and prevent obesity, further efforts to comprehend these basic mechanisms are likely to yield important fundamental and therapeutic information.

**Perspectives**

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. Little is known about the biochemical processes that control hunger and satiety. However, there is evidence that preabsorptive factors are important cofactors in this regulation. Fat ingestion stimulates the secretion of a number of gastrointestinal hormones, including CCK. CCK has been shown to affect the short-term control of food intake in animals and humans during a test meal. Therefore, CCK has been proposed to act as a hormonal satiety signal.

A series of recent studies in humans has shown that peripherally induced CCK can reduce energy intake and modulate subjective appetite sensations. Along this line, the present study illustrates that CCK-8 modulates food intake when given with a preload, supporting a physiological role for the peptide in regulating appetite. However, many questions remain unanswered. Does CCK interact with other gastrointestinal hormones released in response to intraduodenal fat that have been proposed to regulate food intake (glucagon-like peptide-1 and peptide YY)? What is the mechanism of action? Are the effects of CCK mediated by abdominal nerves? CCK dose dependently inhibits gastric emptying; does inhibition of gastric emptying itself reduce food intake, perhaps in association with distension plus oral and intestinal factors? The regul-
luation of food intake is a complex 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 dietary nutrients in the regulation of appetite and body weight and the development of overweight and obesity.

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REFERENCES


