L-Phenylalanine Releases Cholecystokinin (CCK) and Is Associated With Reduced Food Intake in Humans: Evidence for a Physiological Role of CCK in Control of Eating

A.B. Ballinger and M.L. Clark

Exogenous administration of cholecystokinin (CCK) reduces food intake in humans; however, it is not clear if endogenous CCK is a true satiety hormone. The aim of this experiment was to manipulate endogenous release of CCK using l-phenylalanine (L-PA), a potent releaser of CCK, and to measure subsequent food intake. On separate occasions, six normal-weight fasted subjects (four men, two women) were administered 10 g of L-PA, D-PA, or placebo 20 minutes before being presented with a standard meal of known calorie content. Preliminary experiments had shown that peak plasma concentrations of CCK were obtained 20 minutes after administering L-PA. The test meal was given to coincide with this peak. One hundred-millimeter visual analog scales (VAS) to assess hunger, desire to eat, and fullness were completed premeal, postmeal, and at intervals thereafter. Blood was taken before administering PA/placebo immediately premeal and postmeal and stored for measurement of CCK levels by bioassay. Subjects consumed 1,089 ± 86 kcal after L-PA (P < .03) compared with 1,587 ± 174 kcal after placebo and 1,492 ± 126 kcal after D-PA. The reduction in calorie intake after L-PA was associated with a significantly greater sensation of fullness. Basal levels of CCK were 1.10 ± 0.12 pmol/L; 20 minutes after L-PA, CCK levels increased to 5.49 ± 0.83 pmol/L. There was no increase in CCK following D-PA or placebo. Release of CCK by L-PA is associated with a reduction in subsequent food intake, and this suggests that CCK is an important satiety hormone in humans.

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HOLECYSTOKININ (CCK) is present in the central nervous system and endocrine cells of the proximal intestine. It is released into the bloodstream from the gut in response to food in the duodenum and jejunum. In 1973, Gibbs et al reported that intraperitoneal injection of CCK dose-dependently decreased food intake in fasted rats. The satiety response to exogenous CCK has since been shown in numerous species, including the rabbit, pig, monkey, and humans. Animal studies suggest that CCK reduces food intake at least in part by a peripheral action, possibly by binding to receptors on vagal afferent fibers or receptors on the pyloric sphincter and delaying gastric emptying. It is unclear from these experiments if the effects of CCK were pharmacological rather than physiological, since CCK was administered in varying amounts and by different routes. In an attempt to gain additional support for CCK as a satiety peptide, food intake has been measured in response to treatments thought to affect secretion of endogenous CCK. It is suggested that pancreatic trypsin exerts a negative feedback on CCK release, and this is mediated by a CCK-releasing peptide. Trypsin inhibitors increase CCK release presumably by blocking trypsin degradation of the CCK-releasing peptide. In humans, oral administration of trypsin inhibitors before a meal reduces subsequent food intake. However, l-phenylalanine (L-PA), a potent releaser of CCK, is reported as having no effect on subsequent food intake when administered in doses of up to 10 g. However, in all these experiments plasma concentrations of CCK were not measured, and therefore it is not known whether CCK concentrations were actually altered, and if so, what the levels were at the time of the test meal.

In this study, we have manipulated endogenous release of CCK using L-PA and administered a test meal to coincide with the peak plasma concentrations of CCK produced by l-PA. The results are compared with those obtained for placebo and for D-PA, a much weaker stimulant of CCK release.

SUBJECTS AND METHODS

This study was approved by the ethics committee of the City and Hackney Health Authority. A randomized single-blind study was performed in six volunteers (mean age, 30 years and 3 months). Subjects gave their informed consent before participating in the study. All subjects (four men, two women) were within the normal weight range (mean body mass index, 22.2 kg/m²; range, 20.6 to 24.4) for their age, sex, and height.

In all experiments, blood samples were taken from a 21-gauge butterfly cannula placed in the antecubital fossa at the beginning of the experiment. Blood was collected in iced heparinized tubes containing 2,000 KIU/10 mL blood of aprotinin (Trasyrol, Bayer, UK) and immediately centrifuged at 2,000 g for 10 minutes. The plasma obtained was stored at −70°C until assayed for CCK.

Three treatments separated by at least 7 days were performed in each subject. All the experiments were performed at 8:00 AM after an 8-hour fast from midday, at which time subjects had eaten a sandwich (2 slices of bread plus filling). In preliminary experiments, CCK release was determined in response to 10 g of L-PA or D-PA administered orally in 200 mL water (Fig 1). After two baseline blood samples had been taken, subjects were administered either L-PA or D-PA, and blood samples were taken at 10-minute intervals for 1 hour. Based on the results from these experiments (Fig 1), subjects were given a test meal 20 minutes after receiving a premeal of 10 g of either L-PA or D-PA (in 200 mL water) or placebo (200 mL water). The test meal was given to coincide with the peak plasma levels of CCK produced by L-PA. The premeal was administered as a bolus via a nasogastric tube, which was then

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removed immediately. l-PA has a distinctive taste, and by using this method subjects were blinded as to the treatment they were receiving. Blood samples were taken before administration of PA or placebo and then at 10 and 20 minutes after. The test meal (Table 1) was designed to include foods enjoyed by all the volunteers and was presented on a buffet tray so that subjects were free to eat and drink as much as they liked. The components of the meal were individually weighed before and after the meal, and therefore the amount (g) consumed could be calculated. Individual items in the meal were all bought as easily prepared convenience foods in which the calorie content per 100 g was clearly stated on the packaging. Therefore, from the weight of food eaten, the number of calories consumed could be assessed. The food was covered during the experiment to minimize evaporative weight loss from hot food. One hundred-millimeter visual analog scales (VAS) to assess desire to eat, hunger, and fullness were completed before the premeal, immediately before and after the test meal, and at 30-minute intervals thereafter for 90 minutes. VAS were completed in the placebo and L-PA, but not in the D-PA experiment. In addition, subjects were asked to note any side effects from the treatment/placebo.

**Bioassay of CCK**

Plasma concentrations of CCK were measured by a highly sensitive and specific bioassay first described by Liddle et al. This method is based on the ability of CCK to stimulate amylase release from isolated rat pancreatic acini. Plasma is initially extracted and concentrated by adsorption onto octadecylsilica cartridges (C-18 Sep-pak; Millipore, Hertfordshire, UK). CCK is eluted with 1 mL 80% ethanol:0.2% trifluoroacetic acid, and the eluants are dried under nitrogen at 50°C. Plasma extracts are then incubated with isolated rat pancreatic acini, prepared from collagenase digestion of whole rat pancreas, for 30 minutes at 37°C. Amylase released into the incubation medium is assayed using procion yellow-coupled starch as substrate. Amylase release, expressed as a percentage of total amylase content, is compared with the release obtained with CCK-8 standards.

The assay has a detection limit of 1.0 pmol/L. Intraassay and interassay coefficients of variation were 9.4% and 13.4%, respectively.

**Statistical Analysis**

Results are expressed as the mean ± SEM. The energy intake was compared between placebo and PA by the Wilcoxon signed rank test. The scores for hunger, fullness, and desire to eat were also compared at the different time points between treatments by the Wilcoxon signed rank test. P less than .05 was taken as significant.

**RESULTS**

Figure 2 shows the mean energy intakes in each of the three treatment conditions. Subjects consumed 1,089 ± 86 kcal after l-PA, compared with 1,587 ± 174 kcal after placebo and 1,492 ± 126 kcal after D-PA. There was a highly significant difference (P = .03) between the energy intakes after l-PA and placebo; however, energy intake after D-PA was not significantly different from placebo.

The reduced food intake after l-PA was not predicted from the VAS. Before the meal, the scores for hunger and desire to eat were significantly greater in the l-PA group compared with the placebo group (Table 2). After the meal, the scores for hunger and desire to eat were similar between the PA and placebo groups, but subjects felt significantly more full after PA treatment. No subject reported nausea or any other adverse effect with either placebo or PA.

Basal plasma concentrations of CCK were 1.10 ± 0.12 pmol/L; 20 minutes after l-PA and immediately before the test meal, CCK levels increased to 5.39 ± 0.83 pmol/L. There was no significant increase in plasma CCK concentrations following placebo or D-PA.

**Table 1. Components of the Test Meal and Their Calorific Content**

<table>
<thead>
<tr>
<th>Component Food</th>
<th>Calorie Value (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.135 g savory mince</td>
<td>90/100 g</td>
</tr>
<tr>
<td>400 g rice</td>
<td>61.5/100 g</td>
</tr>
<tr>
<td>3 slices gateau</td>
<td>171/slice</td>
</tr>
<tr>
<td>10 savory biscuits</td>
<td>15/biscuit</td>
</tr>
<tr>
<td>60 g cheese</td>
<td>350/100 g</td>
</tr>
</tbody>
</table>

NOTE: The meal was presented on a buffet tray, and subjects were free to eat as much as they liked.

**Fig 1.** Plasma concentrations of CCK were measured after an oral load of l-PA (+) or D-PA (○).

**Fig 2.** Calorie intake after a premeal of l-PA, D-PA, or placebo in six healthy subjects (mean ± SEM). *Significantly (P = .03) different from both other treatments.
These results suggest that CCK inhibits food intake at least in part by its ability to mimic and amplify satiety responses to gastric distension. In this study, 10 g PA contains only 42 kcal. These results therefore support animal data and suggest that CCK is a major satiety hormone in humans.

Previous studies have failed to show a reduction in food intake after similar amounts of L-PA,12,13 and this may relate to the timing of the test meal. In our study, the meal was given to coincide with peak CCK levels produced by L-PA. Also in this study, L-PA was administered with a volume load of 200 mL water, and previous studies have suggested that gastric stimulation interacts with CCK to reduce food intake. Experiments in both animals and humans have shown that gastric loads potentiate and magnify the inhibition of food intake produced by CCK infusions.14,17 A dose of CCK that failed to inhibit food intake when administered alone would suppress food intake when administered in combination with an intragastric volume load. In rats, both intragastric saline loads and celiac artery infusions of CCK-8 increase the firing rate of vagal afferent fibers. In addition, CCK pretreatment significantly enhances the response of these fibers to subsequent gastric loads.18 These results suggest that CCK inhibits food intake at least in part by its ability to mimic and amplify vagal afferent responses to gastric distension. In this study, the reduction in food intake after PA was associated with a greater sensation of fullness compared with placebo, and this finding is in keeping with the proposed action of CCK in reducing food intake.

Intravenous infusions of CCK-8 reduce food intake in both animals and human subjects and suggest that CCK has a role in the control of food intake.5,19 However, in these experiments plasma concentrations of CCK were not measured, and therefore it is not known if the plasma concentrations produced were within the physiological range. In addition, this setting may interfere with eating behavior. In this experiment, by manipulating endogenous release of CCK, we have reproduced postprandial levels with L-PA and thereby mimicked physiological conditions.

Administration of exogenous CCK has been associated with mild nausea in some studies, and it has been suggested that any reduction in food intake is due to nausea rather than to a true satiety effect.5,20,21 In this study none of the subjects reported nausea or any other side effect, and so the reduction in food intake after administration of PA is unlikely to be due to an aversive effect. The results are supportive of CCK as an important factor in the control of eating; however, we cannot rule out other effects of PA. To show that the effect of L-PA was due to CCK release, the experiment would need to be repeated with the addition of a specific and potent CCK antagonist available for human use.

ACKNOWLEDGMENT

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