Effects of cholecystokinin on appetite and pyloric motility during physiological hyperglycemia

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Rayner, C. K., H. S. Park, S. M. Doran, I. M. Chapman, and M. Horowitz. Effects of cholecystokinin on appetite and pyloric motility during physiological hyperglycemia. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G98–G104, 2000.—Recent studies suggest that the interaction between small intestinal nutrient stimulation and the blood glucose concentration is important in the regulation of gastric motility and appetite. The purpose of this study was to determine whether the effects of cholecystokinin octapeptide (CCK-8) on antral and pyloric motility and appetite are influenced by changes in the blood glucose concentration within the normal postprandial range. Seven healthy volunteers were studied on 4 separate days. A catheter incorporating a sleeve sensor was positioned across the pylorus, and the blood glucose was stabilized at either 4 mmol/l (2 days) or 8 mmol/l (2 days). After the desired blood glucose had been maintained for 90 min, an intravenous infusion of either CCK-8 (2 ng·kg⁻¹·min⁻¹) or saline (control) was given for 60 min. Thirty minutes after the infusion began, the catheter was removed and subjects drank 400 ml of water with guar gum before being offered a buffet meal. The amount of food consumed (kcal) was quantified. The order of the studies was randomized and single-blinded. There were fewer antral waves at a blood glucose of 8 than at 4 mmol/l during the 90-min period before the infusions (P < 0.05) and during the first 30 min of CCK-8 or saline infusion (P = 0.07). CCK-8 suppressed antral waves (P < 0.05), stimulated isolated pyloric pressure waves (IPPWs) (P < 0.01), and increased basal pyloric pressure (P < 0.005) compared with control. During administration of CCK-8, basal pyloric pressure (P < 0.01), but not the number of IPPWs, was greater at a blood glucose of 8 mmol/l than at 4 mmol/l. CCK-8 suppressed the energy intake at the buffet meal (P < 0.01), with a significant difference between the two blood glucose concentrations. We conclude that the acute effect of exogenous CCK-8 on basal pyloric pressure, but not appetite, is modulated by physiological changes in the blood glucose concentration.

manometry; hyperglycemia; food intake; hunger

IT IS WELL ESTABLISHED that the interaction of nutrients with the small intestine slows gastric emptying (23), suppresses hunger and subsequent food intake (6, 30, 34), and modifies the perception of other gastrointestinal stimuli, such as gastric distension (13). This interaction is likely to involve gut peptides, which mediate the effects of small intestinal nutrients on satiety (30, 34) and gastric motility (13).

Both marked hyperglycemia (blood glucose of ~15 mmol/l) (36) and elevations of blood glucose within the normal postprandial range (~8 mmol/l) ("physiological" hyperglycemia) (43) slow gastric emptying compared with euglycemia; the motor correlates of this slowing include suppression of antral pressure waves (4, 5) and stimulation of phasic and basal pyloric pressures (15). Hyperglycemia also enhances the perception of gut stimuli, such as gastric distension (21, 22). There is evidence to suggest a synergistic relationship between small intestinal nutrient exposure and the blood glucose concentration; for example, hyperglycemia slows gastric emptying of nutrient but not nonnutrient (salty) drinks (36). Similarly, perceptions of fullness and nausea during intraduodenal lipid infusion are greater during both pathological (20, 21) and physiological (1) hyperglycemia than during euglycemia. Physiological hyperglycemia does not suppress perception of hunger significantly in the absence of nutrients in the small intestine (1, 30).

Cholecystokinin (CCK), which is secreted in response to the presence of nutrients in the small intestine, has an established role in the regulation of both gastric motor function (13, 14, 17, 27, 31, 44) and appetite (2, 3, 32, 33) in humans. CCK analogs slow gastric emptying, whereas CCK antagonists accelerate it (17, 27, 31); the motor correlates of the slowing of gastric emptying by CCK include suppression of antral pressure waves (14, 44) and stimulation of phasic and basal pyloric pressures (14). The effects of intraduodenal lipid on satiety (34) and the perception of gastric distension (13) are mediated, at least in part, by the release of CCK. CCK infusions suppress food intake, and this effect is enhanced when a liquid "preload" is consumed before a meal (2, 18, 33, 37, 40). Although hyperglycemia does not affect the release of CCK in healthy subjects (9), gallbladder emptying in response to exogenous CCK is reduced during both pathological and physiological elevations of the blood glucose concentration (8), suggesting that the effects of CCK on gastrointestinal motility may be modulated by the blood glucose level. The interaction between blood glucose and CCK on gastric motor function, upper gut sensation, and appetite has not been evaluated.

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The aim of the current study was to determine whether the effects of exogenous CCK octapeptide (CCK-8) on pyloric motility, gastrointestinal sensation, and appetite are influenced by changes in the blood glucose concentration within the physiological range. The specific hypotheses were that the stimulation of pyloric motility and suppression of appetite by CCK-8 would be greater at a blood glucose concentration of 8 mmol/l than at 4 mmol/l.

**METHODS**

**Subjects.** Seven healthy volunteers were studied (1 female, 6 males; median age 26 yr, range of 22–37; median body mass index 23 kg/m², range of 20–27). No subject had a history of systemic or gastrointestinal disease, and none was taking medication. All subjects were unrestrained eaters [score of ≤10 on the eating restraint factor of the eating inventory questionnaire (46)]. The study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

**Protocol.** The experimental protocol is summarized in Fig. 1. Each subject participated on 4 separate days. On 2 days, an intravenous infusion of CCK-8 was given; on the other 2 days, saline was given as a control. Both CCK-8 and saline were given while the blood glucose concentration was maintained at either 4 or 8 mmol/l. The order of the studies was randomized, and they were performed in single-blind fashion.

On each study day, subjects attended the laboratory after an overnight fast. A multilumen sleeve-sidehole manometric assembly was passed through an anesthetized nostril and positioned with the sleeve spanning the pylorus. The blood glucose concentration was then stabilized at either 4 or 8 mmol/l using an insulin-glucose clamp (see below) and maintained at the desired level for the duration of the study. When the desired blood glucose concentration was achieved (time \( t = 0 \)), fasting antropyloric pressures were measured for 90 min. An intravenous infusion of either CCK-8 [sincalide (Kinevac), Squibb Diagnostics] or normal saline alone was then commenced and continued for a total of 60 min (i.e., \( t = 90\text{–}150 \text{ min} \)). Thirty minutes after the intravenous infusion was started (\( t = 120 \text{ min} \)), the manometric assembly was removed and a “preload” drink of 400 ml of water containing 4.4 g guar gum (Supercol, Chipmonk, Queensland, Australia) and nonnutritive flavoring was consumed within 5 min. This “preload” was given to distend and then empty from the stomach at a slow rate (25) without stimulating release of endogenous CCK. Five minutes after this (at \( t = 130 \text{ min} \)), a buffet meal was offered, and subjects were allowed to eat as much as they wished for 30 min (7). The buffet meal consisted of a selection of carbohydrate-rich foods (to minimalize release of endogenous CCK) in quantities in excess of what subjects would be expected to eat. Intake of energy and macronutrients was calculated using the DIET/4 program (Xyris Software, Highgate Hill, Queensland, Australia) (7). The CCK-8 or saline infusion ceased 20 min into the meal period (at \( t = 150 \text{ min} \)); however, the insulin-glucose clamp continued until 30 min after the meal (\( t = 190 \text{ min} \)).

**Visual analog questionnaires to assess hunger, fullness, and nausea were completed at 10-min intervals throughout the study, from \( t = 0\text{–}190 \text{ min} \), with the exception of the meal period (45). Venous blood was sampled at the same intervals (including the meal period) for measurement of the blood glucose concentration.

**Stabilization of blood glucose concentrations.** Insulin (Actrapid, Novo Nordisk Pharmaceuticals, NSW, Australia) was added to polygeline solution (Haemaccel, Hoechst Marion Roussel, NSW, Australia) to a concentration of 0.1 U/ml and infused intravenously at a rate of 0.2 mU·kg⁻¹·min⁻¹ throughout each study; in studies conducted at a blood glucose concentration of 4 mmol/l, the rate was increased to 0.8 mU·kg⁻¹·min⁻¹ during the meal period to prevent a postprandial increase in the blood glucose concentration. For studies conducted at a blood glucose of 8 mmol/l, an intravenous bolus of 25% glucose (30–45 ml) was given over 15 min, followed by a variable infusion adjusted according to blood glucose measurements performed using a glucometer (Reflox I1 M, Boehringer Mannheim) (49). In studies performed at a blood glucose concentration of 4 mmol/l, saline was infused at 120 ml/h together with 25% glucose, if required, following an initial saline bolus (40 ml).

**Measurement of antropyloric pressures.** The silicone rubber manometric assembly (Dentsleeve, Adelaide, Australia) incorporated six antral sideholes at 1.5-cm intervals, a 4.5-cm sleeve sensor with two additional pyloric sideholes on the opposite side, and a duodenal sidehole. The tip was allowed to pass into the duodenum by peristalsis, and the transpyloric position of the sleeve was verified by continuous measurement of the transmucosal potential difference between stomach and duodenum, using a sterile saline-filled 20-gauge catheter inserted subcutaneously into the forearm as the reference electrode (24). Intraluminal pressures were recorded at 10 Hz using custom software (DAD, written by G. S. Hebbard using Labview, National Instruments). Automated analysis was performed (MAD, written by C. H. Malbert using Labview) with subsequent exclusion of artifacts by visual inspection of each recording. The following variables were assessed: (1) number, frequency, and amplitude of antropyloric pressure waves (waves of amplitude >10 mmHg in any of the 6 antral sideholes), (2) number, frequency, and amplitude of isolated pyloric pressure waves (IPPWs; waves of amplitude >10 mmHg recorded by the sleeve sensor in the absence of another pressure wave of onset within 5 s of the pyloric wave in the adjacent antral or duodenal sidehole), and (3) basal pyloric pressure (measured as the mean pressure recorded by the sleeve sensor, excluding any phasic waves, in each minute, compared with the baseline pressure measured in the adjacent duodenal sidehole). The change in basal pyloric pressure during CCK-8 or saline infusion was calculated using mean basal pressure in the 15 min preceding the infusion as a baseline.

**Statistical analysis.** Statistical comparisons were made using repeated-measures ANOVA (SuperANOVA, SAS Institute) with the blood glucose concentration, infusion type (CCK-8 or saline), and time as within-subject factors. Visual
analog scores were analyzed for three periods: 0–90 min (before CCK-8 infusion), 90–130 min (during CCK-8 or saline infusion, before the meal), and 160–190 min (postprandial). Direct comparison was also made between the two CCK-8 days by repeated-measures ANOVA to test the specific hypotheses relating to the effects of the blood glucose concentration on the actions of CCK-8, with the blood glucose concentration and time as within-subject factors. Comparisons of number of antral waves at the two blood glucose concentrations in the first 90 min of the study (before CCK-8/saline infusion) were made using Student’s paired t-test (StatView 5, SAS Institute). All data are presented as means ± SE. A P value of <0.05 was considered significant.

RESULTS

Blood glucose concentrations. The mean blood glucose concentrations (Fig. 2) closely approximated the desired range. These concentrations are subsequently referred to as “4 mmol/l” and “8 mmol/l” for simplicity.

Antropyloric pressures. Between t = 0 and t = 90 min (i.e., before intravenous CCK-8 or saline), the number of antral waves was less at a blood glucose of 8 mmol/l than at 4 mmol/l (79.8 ± 10.9 vs. 108.9 ± 12.4, P < 0.05). During the first 30 min of CCK-8 or control infusion (t = 90–120 min) (Fig. 3A), antral waves also tended to be suppressed at a blood glucose of 8 mmol/l compared with that at 4 mmol/l (P = 0.07, blood glucose effect). CCK-8 suppressed antral waves (P < 0.05, infusion effect) with a trend for greater suppression at a blood glucose of 4 mmol/l than at 8 mmol/l (P = 0.10, blood glucose-infusion interaction). Neither the blood glucose nor CCK-8 affected the amplitude of antral waves (data not shown).

CCK-8 stimulated IPPWs compared with saline (P < 0.01, infusion effect) (Fig. 3B); the number and amplitude (data not shown) of IPPWs did not differ between the two blood glucose concentrations during CCK-8 infusion, and there was no interaction between blood glucose and infusion type. Both CCK-8 (P < 0.005, infusion effect) and the higher blood glucose level (P < 0.05, blood glucose effect) were associated with increased basal pyloric pressure (Fig. 3C). Although the interaction between blood glucose and infusion type did not reach significance when comparing all 4 days (P = 0.20), direct comparison of the two CCK-8 days showed that the stimulation of basal pyloric pressure was greater (P < 0.01) at a blood glucose of 8 mmol/l than at 4 mmol/l.

Fig. 2. Blood glucose concentrations in each of the 4 study groups (means ± SE).
There was no effect of CCK-8 or blood glucose on any sensation score in the 30 min after the meal (t = 160–190 min).

Food intake. CCK-8 suppressed energy intake at the buffet meal (P < 0.01, infusion effect), but there was no effect of the blood glucose concentration on energy consumption and no interaction between blood glucose and infusion type (Fig. 5). CCK-8 reduced the intake of each macronutrient class (P < 0.05 for carbohydrate and fat, P < 0.01 for protein, infusion effects); however, blood glucose had no significant effect and no interaction occurred between blood glucose and infusion type. Likewise, comparison of the two CCK-8 days showed no effect of blood glucose on intake of energy or any macronutrient.

**DISCUSSION**

The results of the study indicate that the acute effects of CCK on basal pyloric pressure, but not food intake, are modulated by changes in the blood glucose concentration within the normal postprandial range. These observations are compatible with the concept of a synergistic relationship between the presence of nutrients in the small intestine and the blood glucose concentration in the regulation of gastric emptying mediated, at least in part, by CCK.

We infused CCK-8 at a rate comparable to previous studies investigating its role in appetite regulation (18, 37). Although we did not measure the concentration of CCK in peripheral blood, previous studies suggest that the resulting CCK concentrations would be slightly supraphysiological (18); however, it is possible that local concentrations in the region of the gut are of more importance than peripheral levels. The two blood glucose concentrations in this study were selected to approximate the difference in blood glucose concentrations before and after a carbohydrate-rich meal in normal subjects; the magnitude of this difference is “physiological”, even though the higher blood glucose was maintained for a longer period than normal for the purposes of this model.

Elevation of the blood glucose concentration to postprandial levels (8–10 mmol/l) slows gastric emptying (43), and this is associated with suppression of postprandial antral motility (19) and potentiation of the phasic and tonic pyloric motor responses to duodenal distension (35). We have reported that, in response to duodenal lipid, the frequency of phasic pyloric pressure waves over time is modified by physiological hyperglycemia (more IPPWs initially but a less sustained response at a blood glucose of 8 mmol/l compared with that at 5 mmol/l) (1). Tonic activity of the pylorus is a major determinant of transpyloric flow (48); the observation that blood glucose modulates basal pyloric pressure in response to CCK is therefore potentially important in understanding the physiology of gastric emptying. The blood glucose concentration had no
effect on the number of phasic pyloric pressure waves, but this may be accounted for by the fact that the frequency of IPPWs was already close to maximal after 30 min of CCK-8 infusion even at a blood glucose of 4 mmol/l. The range in basal pyloric pressure is known to be greater than that of IPPWs (11, 15, 16). There is, however, also evidence that phasic and tonic pyloric motor responses may be mediated by different mechanisms, so that differential responses can occur (11, 42).

The site at which the blood glucose modulates the effects of CCK on pyloric motility has not been examined in this study. A direct stimulatory effect on pyloric smooth muscle seems unlikely, given that antral and fundic motility are inhibited by hyperglycemia (4, 5, 19, 21, 22, 41). The role of insulin, secreted in response to hyperglycemia, is controversial (12); the recent observation that euglycemic hyperinsulinemia does not affect gastric emptying in patients with diabetes argues against a significant role (28). Hyperglycemia impairs vagal cholinergic activity (10, 50), but stimulation of the pylorus by CCK-8 is not sensitive to atropine in humans (14) and in rats neural control of pyloric tone is noncholinergic (42). Glucose-responsive neurons have been identified in the rat myenteric plexus (26). The expression of nitric oxide synthase is reduced in the myenteric plexus of diabetic rats (47), but the specific influence of blood glucose on nitrergic pathways in the gut has yet to be examined.

Acute changes in the blood glucose concentration have also been shown to have a major influence on gastrointestinal sensation, with hyperglycemia increasing perceptions of fullness or nausea compared with euglycemia, when combined with gastric or duodenal distension or intraduodenal lipid infusion (20–22, 35). During physiological elevation of the blood glucose concentration, the suppression of hunger by intraduodenal lipid is greater than during euglycemia (1). In contrast, the blood glucose concentration has little effect on hunger in the absence of intestinal nutrients (1, 30). Consistent with this, we observed no significant decrease in hunger or increase in fullness before the meal at a blood glucose of 8 mmol/l compared with 4 mmol/l. Given that intestinal nutrients stimulate the release of CCK, it was reasonable to hypothesize that physiological hyperglycemia may potentiate the effects of CCK on satiety. This would also explain why physiological hyperglycemia has only a modest effect, if any, on food consumption without a prior nutrient stimulus (7, 30), as the release of CCK would only begin some minutes into the meal.

Our results confirm that exogenous administration of CCK-8 suppresses food intake and indicate that the magnitude of this suppression is similar at both blood glucose levels; there is no evidence for any interaction between blood glucose and the effects of CCK on appetite. Lam et al. (29) have recently reported that marked hyperglycemia (15 mmol/l) reverses the satiating effects of both intraduodenal lipid and intravenous CCK-33, resulting in increased hunger scores compared with euglycemia; however, both the low rate of the lipid infusion (1 g/h or 0.15 kcal/min) and the pathological degree of hyperglycemia limit the physiological relevance of these observations. Interestingly, we were unable to demonstrate any effect of CCK-8 on subjective ratings of hunger, despite the fact that CCK-8 diminished caloric consumption by almost 30%. An effect of intravenous CCK-33 on hunger scores in the absence of gastric distension or intestinal nutrients has been shown by one group, but the difference from intravenous saline was small and a relatively large number of subjects was studied (32). Another factor, in addition to gastric distension, appears to be required for CCK to induce substantial satiety; such a factor might be the activation of intestinal nutrient receptors, the release of gut hormones in addition to CCK, and/or the presence of the products of digestion in the portal circulation.

Factors other than modulation of the actions of CCK must also be considered to explain the synergy we have previously observed between physiological hyperglycemia and intestinal lipid (1). In animals, satiety is influenced by the length of intestinal contact with the nutrient and, possibly, by the region of small intestine exposed to nutrients (38, 39). In previous studies, the effects of intraduodenal lipid infusion on perception of hunger were only evident 45 min after the infusion began (1), suggesting that mechanisms triggered by the presence of lipid in the distal small intestine may be important.

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