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Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake, and antropyloroduodenal motility in healthy men

IxcHEL M. BRENNAN,1 Kate L. Feltrin,1 Michael Horowitz,1 Andre J. P. M. Smout,2 James H. Meyer,1 Judith Wishart,1 and Christine Feinle-Bisset1

1Department of Medicine, University of Adelaide, Royal Adelaide Hospital, Adelaide, South Australia, Australia; and 2Department of Gastroenterology, University Hospital, Utrecht, The Netherlands

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Brennan, I. M., Kate L. Feltrin, Michael Horowitz, Andre J. P. M. Smout, James H. Meyer, Judith Wishart, and Christine Feinle-Bisset. Evaluation of interactions between CCK and GLP-1 in their effects on appetite, energy intake, and antropyloroduodenal motility in healthy men. Am J Physiol Regul Integr Comp Physiol 288: R1477–R1485, 2005. —There is evidence that CCK and glucagon-like peptide-1 (GLP-1) mediate the effects of nutrients on appetite and gastrointestinal function and that their interaction may be synergistic. We hypothesized that intravenous CCK-8 and GLP-1 would have synergistic effects on appetite, energy intake, and antropyloroduodenal (APD) motility. Nine healthy males (age 22 ± 1 yr) were studied on four separate days in a double-blind, randomized fashion. Appetite and APD pressures were measured during 150-min intravenous infusions of J) isotonic saline (control), 2) CCK-8 (1.8 pmol·kg⁻¹·min⁻¹), 3) GLP-1 (0.9 pmol·kg⁻¹·min⁻¹), or 4) both CCK-8 (1.8 pmol·kg⁻¹·min⁻¹) and GLP-1 (0.9 pmol·kg⁻¹·min⁻¹). At 120 min, energy intake at a buffet meal was quantified. CCK-8, but not GLP-1, increased fullness, decreased desire to eat and subsequent energy intake, and increased the number and amplitude of isolated pyloric pressure waves and basal pyloric pressure (P < 0.05). Both CCK-8 and GLP-1 decreased the number of antral and duodenal pressure waves (PWs) (P < 0.05), and CCK-8+GLP-1 decreased the number of duodenal PWs more than either CCK-8 or GLP-1 alone (P < 0.02). This was not the case for appetite or isolated pyloric PWs. In conclusion, at the doses evaluated, exogenously administered CCK-8 and GLP-1 had discrepant effects on appetite, energy intake, and APD pressures, and the effects of CCK-8+GLP-1, in combination, did not exceed the sum of the effects of CCK-8 and GLP-1, providing no evidence of synergism.

cholecystokinin; glucagon-like peptide-1

FOOD INGESTION TRIGGERS a number of stimuli within the gastrointestinal tract that modulate motility, secretion, and appetite, including gastric distension (21), the presence of nutrients in the small intestine (4, 6, 8), and the release of gastrointestinal hormones, including CCK and glucagon-like peptide-1 (GLP-1) (20, 23). Although some inconsistencies exist in regard to the roles of CCK and GLP-1 in appetite regulation, there is persuasive evidence that they both modulate the effects of nutrients on gastrointestinal function and appetite (9, 10, 26, 38, 42). Both CCK and GLP-1, when administered intravenously to healthy subjects, appear to have comparable effects on appetite and energy intake, increasing the perception of fullness and decreasing hunger and energy intake (9, 22, 42). CCK and GLP-1 also modulate gastroduodenal contractile activity and slow gastric emptying (1, 10, 38); the latter may contribute to the suppression of energy intake (19, 42). Studies using the CCK-A antagonist loxiglumide have established that the effects of CCK on gastric emptying and appetite are mediated through the CCK-A receptor in humans (2, 11); the effects of GLP-1 antagonists on gastric emptying and appetite have not been evaluated in humans.

Although ingestion of a meal is known to trigger a rise in blood concentrations of CCK and GLP-1 within ~15 min (20, 23), there is little information relating to any possible interaction in the effects of these two hormones (14). This knowledge is potentially important for an understanding of the mechanisms underlying food intake regulation, with evidence that the development of obesity is associated with different patterns of gastrointestinal hormone release (32, 43). A number of studies have investigated possible interactions between other gastrointestinal stimuli (6, 12, 15, 21). For example, gastric distension and CCK may have synergistic (i.e., the combined effect of the two stimuli is greater than the sum of their individual effects), rather than additive (i.e., the combined effect of the two stimuli equals the sum of their individual effects), effects (21). In a previous study the combination of gastric distension (with 300 ml water) and intravenous CCK-8 [dose: 112 ng/ml (~102 pmol/min) for 23 min] reduced food intake (in g) in healthy male and female subjects much more (by 200 g) than either CCK-8 (96 g) or distension (3 g) alone (21). Observations derived from a recent study in our laboratory (8) are consistent with the concept of an interaction in the effects of CCK and GLP-1. This study investigated the effects of duodenal infusion of decaenoic acid, a fatty acid with 10 carbon atoms (C10), and lauric acid, a fatty acid with 12 carbon atoms (C12), on appetite, antropyloroduodenal (APD) motility, and CCK and GLP-1 release in healthy subjects. C12 was shown to decrease energy intake and increase pyloric pressures compared with both C10 and control. C10 and C12 also differed in their effects on CCK and GLP-1 release in that C12 increased plasma CCK and GLP-1, whereas C10 increased plasma CCK, albeit to a lesser extent than C12, and had no effect on GLP-1. It is, therefore, plausible that the combined actions of CCK and...
GLP-1 (possibly with other gut peptides) were responsible for the more potent effects of C12 on appetite and APD motility. A study investigating the effects of intravenous CCK-33 and GLP-1, alone and in combination, on energy intake and appetite has recently been published (14). In this study, intravenous infusion of both CCK-33 and GLP-1 resulted in a nonsignificant decrease in the perception of hunger before a meal. In contrast, when CCK-33 and GLP-1 were infused together, there was a reduction in hunger compared with CCK-33 or GLP-1 alone. However, surprisingly, the combination of CCK-33 and GLP-1 did not reduce energy intake any more than either CCK-33 or GLP-1 alone, rather, the effect was infra-additive. There is currently no information about the effects of the combination of CCK and GLP-1 on APD motility.

The aims of the current study were therefore to determine the effects of intravenous CCK-8 and GLP-1, given alone or in combination, on appetite, energy intake, and APD motility in healthy male subjects. We evaluated the hypothesis that intravenous infusions of CCK and GLP-1 would have synergistic effects on these parameters, specifically that the suppression of appetite and energy intake and modulation of APD motility would be greater when the two peptides are administered together compared with alone.

SUBJECTS AND METHODS

Subjects

Nine healthy male subjects, aged 22 ± 1 yr (range 18–27 yr), were recruited according to guidelines established by the Royal Adelaide Hospital Human Ethics Committee. The number of subjects was based on power calculations derived from our previous studies (7, 26). All subjects were required to be of normal body weight for their height (body mass index 23 ± 0.5 kg/m², range 20–25.2 kg/m²; body weight 75 ± 0.2 kg, range 81.5–63.5 kg) and unrestrained eaters [score ≤ 12 on the eating restraint component of the Three Factor Eating questionnaire (40)]. The following exclusion criteria also applied: 1) significant gastrointestinal disease, symptoms, or surgery; 2) current use of medication known to affect gastrointestinal function, appetite, or body weight; 3) significant cardiovascular or respiratory disease; 4) allergy to local anesthetic; 5) cigarette smoking or an alcohol intake in excess of 20 g/day; 6) abnormal liver function by routine biochemistry tests. The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, and all subjects provided written informed consent before their inclusion.

Protocol

Each subject attended the laboratory on four occasions, each separated by 3–10 days, where they received, in randomized, double-blind fashion, intravenous infusions of 1) isotonic saline (control), 2) CCK-8 at 1.8 pmol·kg⁻¹·min⁻¹ (2 ng·kg⁻¹·min⁻¹), 3) GLP-1 at 0.9 pmol·kg⁻¹·min⁻¹ (both from Merck Biosciences, Läufelfingen, Switzerland), or 4) both 1.8 pmol·kg⁻¹·min⁻¹ CCK-8 and 0.9 pmol·kg⁻¹·min⁻¹ GLP-1 (CCK-8+GLP-1). The peptides were dissolved in 0.9% sterile saline. The doses of CCK-8 and GLP-1 were selected on the basis of previous studies that indicated that they had submaximal effects on gastric emptying, energy intake, and APD pressures, while resulting in physiological plasma concentrations (26, 29, 34, 38). In all studies, appetite, energy intake, and APD motility were evaluated.

Subjects attended the laboratory at 0830 after fasting from solid and liquid food from 2200 h the previous night. A 16-channel manometric catheter (Dentsleeve; Adelaide, Australia), to measure pressures in the APD region, was inserted through an anesthetized nostril and allowed to pass through the pylorus into the duodenum by peristalsis (4). The catheter was positioned with six side holes in the antrum (channels 1–6), a 4.5-cm sleeve sensor (channel 7) with two side holes on the back of the sleeve (channels 8 and 9) across the pylorus, and 7 side holes in the duodenum (channels 10–16). The distance between side holes was 1.5 cm. The position of the catheter was maintained by measurement of the transmucosal potential difference (TMPD) between the most distal antral (channel 6, approximately ~40 mV) and the most proximal duodenal (channel 10, ~0 mV) channel (17) with a cannula filled with sterile saline and placed subcutaneously in the left forearm as a reference electrode. All channels were perfused with degassed, distilled water at 0.15 ml/min, with the exception of the two TMPD channels, which were perfused with degassed 0.9% saline. Intravenous cannulas were placed in each arm for intravenous infusion and blood sampling, respectively.

Once the catheter was in place, fasting motility was monitored until the occurrence of a phase III of the interdigestive migrating motor complex, and during a phase of motor quiescence (3), a “baseline” (t = 0 min to 15 min) blood sample was taken and a visual analog scale (VAS) questionnaire assessing perceptions of appetite was administered. At t = 0 min, 1) control, 2) CCK-8, 3) GLP-1, or 4) CCK-8+GLP-1 infusion was commenced and continued for 150 min. During the infusion blood samples were obtained and VAS was completed at regular intervals. At t = 120 min, subjects were extubated and immediately offered a standardized, cold buffet-style meal (8). The meal consisted of bread, cold meats, cheese, lettuce, tomato, cucumber, mayonnaise, butter, apple, banana, yogurt, chocolate custard, fruit salad, iced coffee, orange juice, and water. The amount of food offered was in excess of what a subject could be expected to consume. Subjects were allowed up to 30 min to consume their meals and instructed to eat until comfortably full. At t = 150 min the infusion was stopped, a final blood sample was taken, and a VAS was administered. Subjects were then monitored for a further 30 min and, after removal of the intravenous cannulas, allowed to leave the laboratory.

Measurements

Appetite and energy intake. Appetite ratings (fullness, desire to eat) were assessed by validated VAS (30). Nausea and bloating were also quantified. Each VAS consisted of a 100-mm horizontal line, on which 0 represented “sensation not felt at all” and 100 “sensation felt the greatest.” The subject placed a vertical mark along the line to indicate the strength of each sensation every 10 min between 0 and 30 min, every 15 min until t = 60 min, and every 30 min until t = 150 min. The energy (kJ) and amount (g) consumed at the buffet meal, including macronutrient distribution (% energy from fat, carbohydrate, and protein), were evaluated with the software program Foodworks 3.01 (Xyris Software, Highgate Hill, Australia; Ref. 8).

APD pressures. Manometric pressures recorded between −15 and 120 min were digitized on a computer-based system running commercially available software (HAD; Assoc. Prof. G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia) and stored for subsequent analysis. APD pressures were analyzed for 1) number and amplitude of pressure waves (PWs) in the antrum and duodenum, 2) number and amplitude of isolated pyloric pressure waves (IPPWs), 3) basal pyloric pressure (pyloric “tone”), and 4) pressure wave sequences (PWSs) (8). PWs in the antrum, pylorus, and duodenum were defined by an amplitude of ≥10 mmHg, with a minimum interval of 15 s between peaks for antral and pyloric waves and 3 s for duodenal waves (36), and analyzed with custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, Netherlands). Basal pyloric pressures were calculated by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve (18), using custom-written software (MAD; Prof. C.-H. Malbert, Institut National de la Recherche Agronomique, Rennes, France). PWs in the antrum, pylorus, and duodenum were considered...
related and defined as PWSs if their rate of travel between side holes was between 9 and 160 mm/s (36). PWSs were characterized according to the distance traveled, i.e., over at least 2 (1.5 to <3 cm), 3 (3 to <4.5 cm), 4 (4.5 to <6 cm), 5 (6 to <7.5 cm), 6 (7.5 to <9 cm), 7 (9 to <10.5 cm) 8 (10.5 to <12 cm), 9 (12 to <13.5 cm), 10 (13.5 to <15 cm), 11 (15 to <16.5 cm), 12 (16.5 to <18 cm), 13 (18 to <19.5 cm), 14 (19.5 to <21 cm), and 15 (21 to <22.5 cm) channels, and expressed as total number of waves with custom-written software (Gastrointestinal Motility Unit, Utrecht, Netherlands).

**Plasma CCK and GLP-1 Concentrations**

Ten-milliliter venous blood samples, for evaluation of plasma CCK and GLP-1, were collected in chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasyloc; Bayer Australia, Pymble, Australia) per liter of blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at –70°C until assayed. CCK-8 and GLP-1 were measured on the blood samples obtained at t = 0, 10, 30, 120, 150, and 180 min.

Plasma CCK (pmol/l) was determined by radioimmunoassay after ethanol extraction (26). The antibody used (C258; lot 105H4852, Sigma Chemical, St Louis, MO) binds to all CCK peptides containing the sulfated tyrosine residue in position 7, shows a 26% cross-reactivity with unsulfated CCK-8 and <2% cross-reactivity with human gastrin, and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9% and the interassay CV was 27%, with a sensitivity of 2.5 pmol/l.

Plasma GLP-1 (pmol/l) was measured by radioimmunoassay (44). The antibody, supplied by Professor S. R. Bloom (Hammersmith Hospital, London, UK), did not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1(7–36) amide. Intra-assay CV was 17% and interassay CV was 18%, with a sensitivity of 1.5 pmol/l.

**Statistical Analysis**

Baseline (“0”) was calculated as the mean of values obtained at t = −15 and 0 min for VAS and plasma hormone concentrations and between t = −15 and 0 min for the total number and amplitude of antral and duodenal PWs, IPPWs, mean basal pyloric pressures, and total number of PWSs. The number and amplitude of antral and duodenal PWs were expressed as mean values during the first 120 min of the infusion period. IPPWs, basal pyloric pressure, and PWSs were expressed as mean values for 15-min intervals between −15 and 120 min (i.e., 0−15, 15–30, ..., 105−120 min). PWSs were expressed as the total number of waves traveling over between 2 and 15 channels during the first 120 min of the infusion period. All data, with the exception of plasma CCK and GLP-1 concentrations, were expressed as changes from baseline. VAS, plasma hormone concentrations, IPPWs, basal pyloric pressures, and PWSs were analyzed by repeated-measures ANOVA with time (t = 0, 10, 20, 30, ..., 120 min, or t = 0−15, 15–30, ..., 105–120 min, see above) and treatment as factors. One-way ANOVA was used to analyze the effect of treatment on the number and amplitude of antral and duodenal PWs, as well as energy intake. Post hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. In accordance with correct statistical practice, significant effects are reported as time by treatment interaction, treatment effect, and/or time effect, in this hierarchy, i.e., by definition, when a treatment effect is reported, no time by treatment interaction is evident. Plasma CCK-8 and GLP-1 concentrations at 120, 150, and 180 min were compared with Student’s paired t-test. Statistical significance was accepted at P < 0.05, and data are presented as means ± SE.

**RESULTS**

All subjects tolerated the experimental conditions well.

**Appetite**

**Perceptions.** There was a significant effect of treatment on scores for desire to eat (treatment effect: P = 0.037) (Fig. 1A). CCK-8 decreased scores for desire to eat compared with both control (CCK-8 vs. control: P = 0.027) and GLP-1 (CCK-8 vs. GLP-1: P = 0.008), whereas there was no difference between
GLP-1 and control. There was no significant difference between CCK-8+GLP-1 and CCK-8 or GLP-1. There was a significant effect of time on scores for desire to eat (time effect: \( P = 0.009 \)). Scores for desire to eat decreased during the first 10 min of the CCK-8 infusion and did not change over the subsequent 110 min.

There was a treatment by time interaction for scores for fullness (treatment * time: \( P = 0.046 \)) (Fig. 1B). CCK-8 increased fullness scores between 10 and 60 min of the infusion period compared with control (CCK-8 vs. control: \( P < 0.001 \)), GLP-1 (CCK-8 vs. GLP-1: \( P < 0.001 \)), and CCK-8+GLP-1 (CCK-8 vs. CCK-8/GLP-1: \( P < 0.001 \)). At 90 min, there was a trend for CCK-8 to increase fullness score compared with control and GLP-1 (\( P = 0.09 \)), and CCK-8 increased fullness score compared with CCK-8+GLP-1 (CCK-8 vs. CCK-8+GLP-1: \( P = 0.008 \)).

There was a significant effect of treatment on scores for nausea (treatment effect: \( P = 0.026 \)) (Fig. 1C). CCK-8 increased nausea scores compared with control (CCK-8 vs. control: \( P = 0.028 \)), whereas there was no difference between GLP-1 and control or between CCK-8 and GLP-1. Although there was no difference between CCK-8+GLP-1 and CCK-8, there was a trend for CCK-8+GLP-1 to increase nausea scores to a greater extent than GLP-1 (\( P = 0.07 \)).

Energy intake. There was a significant effect of treatment on energy intake (treatment effect: \( P = 0.003 \)) (Fig. 2). CCK-8 decreased energy intake compared with both control (CCK-8 vs. control: \( P = 0.002 \)) and GLP-1 (CCK-8 vs. GLP-1: \( P = 0.001 \)), whereas there was no difference between GLP-1 and control. CCK-8+GLP-1 decreased energy intake compared with GLP-1 (CCK-8+GLP-1 vs. GLP-1: \( P = 0.001 \)) but not CCK-8. Similarly, there was a treatment effect on the amount (g) eaten at the buffet meal (treatment effect: \( P = 0.002 \)). CCK-8 decreased the amount eaten compared with both control (CCK-8 vs. control: \( P = 0.001 \)) and GLP-1 (CCK-8 vs. GLP-1: \( P = 0.003 \)); however, there was no difference between GLP-1 and control. CCK-8+GLP-1 decreased the amount consumed compared with GLP-1 (CCK-8+GLP-1 vs. GLP-1: \( P = 0.03 \)), but the effect did not differ from that of CCK-8 (data not shown).

There was no difference in macronutrient distribution, i.e., the percentage of energy from fat, carbohydrates, and protein consumed at the buffet meal, between treatments (fat: control 31.4%, CCK-8 31.1%, GLP-1 34.5%, CCK-8+GLP-1 32.2%; carbohydrates: control 44.7%, CCK-8 46.4%, GLP-1 42.8%, CCK-8+GLP-1 44.8%; protein: control 23.6%, CCK-8 22.9%, GLP-1 23.3%, CCK-8+GLP-1 23.1%).

ADP Pressures

Antral pressures. There was a significant effect of treatment on the number of antral PWs (treatment effect: \( P = 0.001 \)) (Fig. 3A). Both CCK-8 and GLP-1 decreased the number of PWs compared with control (CCK-8 vs. control: \( P = 0.001 \)), GLP-1 vs. control: \( P = 0.002 \), with no difference between CCK-8 and GLP-1. Although the mean number of antral waves was less with CCK-8+GLP-1 than with CCK-8 or GLP-1 alone, this difference was not significant.

There was an effect of treatment on the amplitude of antral PWs (treatment effect: \( P = 0.002 \)) (Fig. 3B). Both CCK-8 and GLP-1 decreased the amplitude of antral PWs compared with control (CCK-8 vs. control: \( P = 0.001 \), GLP-1 vs. control: \( P = 0.001 \)), with no difference between CCK-8 and GLP-1. Although the mean amplitude of antral waves was lower with CCK-8+GLP-1 compared with CCK-8 or GLP-1 alone, this difference was not significant.

Pyloric pressures. Basal pressure (tone). There was a significant effect of treatment on basal pyloric pressure (treatment effect: \( P = 0.003 \)) (Fig. 4A). CCK-8 increased basal pyloric pressure compared with both control (CCK-8 vs. control: \( P = 0.001 \)) and GLP-1 (CCK-8 vs. GLP-1: \( P = 0.003 \)), with no difference between GLP-1 and control. CCK-8+GLP-1 also increased basal pyloric pressure compared with control (CCK-8+GLP-1 vs. control: \( P = 0.018 \)), with no significant difference between CCK-8+GLP-1 and CCK-8. There was a significant effect of time on basal pyloric pressure (time effect: \( P = 0.035 \)). Both CCK-8 and CCK-8+GLP-1 caused a marked increase in basal pyloric pressure during the first 15 min of infusion before gradually decreasing to reach baseline levels by 120 min.

Phasic pressures. There was a treatment by time interaction for the number of IPPWs (\( P = 0.01 \)) (Fig. 4B). CCK-8 increased the number of IPPWs compared with both control (CCK-8 vs. control: \( P < 0.006 \)) and GLP-1 (CCK-8 vs. GLP-1: \( P < 0.01 \)) over the entire infusion period (\( t = 0-120 \) min), whereas there was no difference between GLP-1 and control. The combination of CCK-8+GLP-1 increased the number of IPPWs compared with control between 0 and 30 min and between 60 and 120 min (CCK-8+GLP-1 vs. control: \( P < 0.03 \)). In contrast, the number of IPPWs was lower during CCK-8+GLP-1 between 15 and 60 min and between 75 and 90 min of the infusion period compared with CCK-8 (CCK-8+GLP-1 vs. CCK-8: \( P < 0.03 \)) but higher between 0 and 45 min and between 60 and 75 min compared with GLP-1 (CCK-8+GLP-1 vs. GLP-1: \( P < 0.02 \)).

There was an effect of treatment on the amplitude of IPPWs (treatment effect: \( P = 0.01 \)) (Fig. 4C). CCK-8 increased the amplitude of IPPWs compared with control (CCK-8 vs. control: \( P = 0.007 \)) but not GLP-1; there was no difference between GLP-1 and control. CCK-8+GLP-1 also increased the amplitude of IPPWs compared with control (CCK-8+GLP-1
CCK AND GLP-1 INTERACTIONS, APPETITE, AND GUT MOTILITY

Fig. 3. Number (A) and amplitude (B) of antral pressure waves (PWs) during intravenous control, CCK-8, GLP-1, and CCK-8+GLP-1 infusion. A: CCK-8 and GLP-1 decreased the number of PWs compared with control, with no difference between CCK-8 and GLP-1. Although the mean number of antral waves was lower with CCK-8+GLP-1 than with CCK-8 or GLP-1 alone, this difference was not significant. Treatment effect: P = 0.001. *CCK-8, GLP-1, and CCK-8+GLP-1 vs. control: P < 0.002. B: CCK-8 and GLP-1 decreased the amplitude of antral waves compared with control, with no difference between CCK-8 and GLP-1. There was no difference between CCK-8+GLP-1 and CCK-8 or GLP-1 alone. Treatment effect: P = 0.002. *CCK-8, GLP-1, and CCK-8+GLP-1 vs. control: P < 0.002. Data are means ± SE (n = 9).

Duodenal pressures. There was a significant effect of treatment on the number of duodenal PWs (treatment effect: P = 0.001) (Fig. 5). Both CCK-8 and GLP-1 decreased the number of duodenal PWs compared with control (CCK-8 vs. control: P = 0.001; GLP-1 vs. control: P = 0.001), with no difference between CCK-8 and GLP-1. CCK-8+GLP-1 reduced duodenal PWs more than either CCK-8 (CCK-8+GLP-1 vs. CCK-8; P = 0.025) or GLP-1 (CCK-8+GLP-1 vs. GLP-1; P = 0.001) alone. There was a trend for the amplitude of duodenal PWs to differ between study conditions (treatment effect: P = 0.07), so that the amplitude tended to be lower after CCK-8, GLP-1, and CCK-8+GLP-1 compared with control (data not shown).

Pressure wave sequences. There was a significant effect of treatment on the number of PWs traveling over two (i.e., 1.5 to < 3 cm; P = 0.029), three (i.e., 3 to < 4.5 cm; P = 0.035), four (i.e., 4.5 to < 6 cm; P = 0.004), five (i.e., 6 to < 7.5 cm; P = 0.014), six (i.e., 7.5 to < 9 cm; P = 0.03), seven (i.e., 9 to < 10.5 cm; P = 0.008), eight (i.e., 10.5 to < 12 cm; P = 0.001) and nine (i.e., 12 to < 13.5 cm; P = 0.001) channels (Fig. 6).

GLP-1 reduced duodenal pressures. There was a significant effect of time on the amplitude of IPPWs (time effect: P < 0.001). GLP-1 reduced duodenal pressure waves was lower with CCK-8 GLP-1 than with CCK-8 or GLP-1 alone, this difference was not significant. Treatment effect: P = 0.001. *CCK-8, GLP-1, and CCK-8+GLP-1 vs. control: P < 0.002. *CCK-8 vs. GLP-1; P = 0.002. Data are means ± SE (n = 9).

CCK-8 increased basal pyloric pressure compared with control and GLP-1, with no difference between GLP-1 and control. There was no difference between CCK-8 and GLP-1. There was no difference between CCK-8+GLP-1 and CCK-8 or GLP-1 alone. Treatment effect: P < 0.002. *CCK-8, GLP-1, and CCK-8+GLP-1 vs. control: P < 0.002. Data are means ± SE (n = 9).

Fig. 4. Basal pyloric pressure (A) and number (B) and amplitude (C) of isolated pyloric pressure waves (IPPWs), occurring during 15-min intervals, during intravenous control, CCK-8, GLP-1, and CCK-8+GLP-1 infusion. A: CCK-8 increased basal pyloric pressure compared with control and GLP-1, with no difference between GLP-1 and control or between CCK-8+GLP-1 and CCK-8. Treatment effect P = 0.005. *CCK-8 vs. control; P = 0.001; *CCK-8 vs. GLP-1; P = 0.003. B: there was a treatment by time interaction for the number of IPPWs (P = 0.01). CCK-8 increased the number of IPPWs compared with both control (CCK-8 vs. control: P < 0.006) and GLP-1 (CCK-8 vs. GLP-1: P < 0.01), whereas there was no difference between GLP-1 and control. In contrast, the number of IPPWs was less during CCK-8+GLP-1 between 15 and 60 min and between 75 and 90 min of the infusion period compared with CCK-8 (CCK-8+GLP-1 vs. CCK-8: P < 0.03). CCK-8+GLP-1 increased the number of IPPWs compared with GLP-1 (CCK-8+GLP-1 vs. GLP-1: P < 0.02) between 0 and 45 min and between 60 and 75 min. *CCK-8 vs. control; P = 0.006; *CCK-8+GLP-1 vs. CCK; P < 0.03; *CCK-8+GLP-1 vs. GLP-1: P < 0.02. C: CCK-8 increased the amplitude of IPPWs compared with control, but not GLP-1, with no difference between GLP-1 and control. There was no difference between CCK-8+GLP-1 and CCK-8. Treatment effect: P = 0.01. *CCK-8 vs. control: P = 0.007. Data are means ± SE (n = 9).
GLP-1, but not CCK-8, decreased the number of waves that traveled over two channels compared with control (GLP-1 vs. control; \( P = 0.044 \)), although there was no difference between CCK-8 and GLP-1. Both CCK-8 and GLP-1 decreased the number of waves that traveled over three, four, five, seven, eight, and nine channels compared with control (CCK-8 vs. control; \( P < 0.03 \); GLP-1 vs. control; \( P < 0.02 \)), with no difference between CCK-8 and GLP-1. CCK-8 decreased the number of waves that traveled over six channels (CCK-8 vs. control; \( P = 0.03 \)), whereas GLP-1 had no effect. There was no significant difference between CCK-8+GLP-1 and either CCK-8 or GLP-1 for the number of PWSs traveling over two to nine channels. PWSs traveling over 10 and more channels were not analyzed statistically, as they were very infrequent (a total of 18 waves traveled over 10–15 channels, 7 during the control infusion, 3 during CCK-8, 5 during GLP-1, and 3 during CCK-8+GLP-1).

**Discussion**

The observations derived from this study indicate that intravenous administration of CCK-8 and GLP-1, in the doses that were evaluated, have discrepant effects on appetite, energy intake, and APD motility in healthy, young men. Infusion of CCK-8 decreased perceptions of appetite, energy intake, the number of antral and duodenal PWSs, and the number of PWSs.
Clarification as to whether there is an interaction between CCK-8 and GLP-1 is relevant for an understanding of the mechanisms regulating energy intake and gastrointestinal motility and, potentially, the pathogenesis of obesity. Intravenous infusion of CCK-8 has been reported in the majority of studies to increase fullness, suppress hunger, and inhibit energy intake in healthy humans (22, 26), consistent with our observations. Although it could be argued that the plasma CCK concentrations resulting from the infusion in this study may be moderately supraphysiological (13, 37), they are comparable with the concentrations observed after a meal in our previous studies where plasma CCK-8 was measured with an identical assay (25, 41). Although the majority of studies have reported that intravenous infusion of GLP-1 decreases perceptions of appetite and reduces energy intake (9, 16, 42), some have failed to demonstrate any appetite-suppressant effect (24, 27). A meta-analysis suggests that the effects of exogenous administration of GLP-1 on appetite and energy intake, although significant, are also modest (42). Furthermore, in some studies nausea and vomiting occurred after administration of relatively high doses of GLP-1 (~4.5 nmol/kg body wt) (28, 35). The dose of GLP-1 (0.9 pmol·kg⁻¹·min⁻¹) we used was comparable to that in previous studies (9, 14, 24) and resulted in slightly supraphysiological plasma levels, compared with those observed in response to a meal or duodenal nutrient infusion (5, 31). Furthermore, plasma GLP-1 levels at 180 min (i.e., after the meal and 30 min after the cessation of the GLP-1 infusion) were not significantly different from those at 120 min, and no nausea occurred in any subjects. Clearly, studies using specific GLP-1 antagonists (39) are indicated to further establish, or refute, a physiological role for GLP-1 in the regulation of energy intake in humans.

Compared with individual infusions of CCK-8 and GLP-1, the combination of CCK-8+GLP-1 did not suppress desire to eat or enhance fullness any further. This observation is apparently at odds with the outcome of a recent study reporting that concurrent intravenous administration of CCK-33 and GLP-1 reduced hunger more than either CCK-33 or GLP-1 alone (14). The reason for this discrepancy is unclear; however, it should be recognized that the type and dose of CCK used in the study by Gutzwiller et al. (CCK-33, 0.2 pmol·kg⁻¹·min⁻¹; Ref. 14) are different from our study. Moreover, in their study, appetite perceptions were measured with a category scale and not, as in our investigation, a true VAS. An apparent anomaly in their study (14) was that the combination of CCK-33+GLP-1 did not suppress energy intake more than infusion of CCK-33 or GLP-1 alone; rather, there was an infra-additive reduction in energy intake, the magnitude of this reduction was comparable to that induced by CCK-8 alone.

Intravenous administration of both CCK and GLP-1 have been reported, in animals and humans, to suppress antral and duodenal PWSs and stimulate pyloric pressures (1, 10, 38); these effects are associated with the slowing of gastric emptying (1, 11, 33). In our study, CCK-8 infusion reduced the number of antral and duodenal PWSs and the amplitude of antral PWSs and PWSs increased the number and amplitude of IPPWs. GLP-1 also reduced the number of antral and duodenal PWSs and the amplitude of antral PWSs and PWSs but, perhaps surprisingly, had no effect on IPPWs. Hence, a dose of GLP-1...
that did not affect appetite or energy intake or stimulate pyloric pressures had effects on antral and duodenal PWs comparable to a dose of CCK-8 that did suppress both appetite and energy intake and stimulated both phasic and tonic pyloric pressures. It could therefore be argued that discrepant effects on pyloric motility may account for the observed differences in the effects of CCK-8 and GLP-1 on energy intake. Although our data do not suggest that there is a close relationship between changes in APD pressures and appetite perceptions, the relationship between pyloric motility and appetite and energy intake clearly warrants evaluation, particularly as a recent study has reported that electrical stimulation of the pylorus decreases energy intake in dogs (45). Certainly, our data indicate that there are different thresholds for effects of GLP-1 on gastrointestinal motility and energy intake.

Our study represents the first evaluation of the combined effects of CCK-8 and GLP-1 on APD motility. CCK-8 + GLP-1 significantly reduced the number of duodenal PWs compared with CCK-8 or GLP-1 alone, and mean values for antral PWs were also lower. However, although it may be considered that the effects of CCK-8 + GLP-1 on APD motility are greater than those of either CCK-8 or GLP-1 alone, there was no evidence of synergism. It should be recognized that the doses of CCK-8 and GLP-1 used may have exerted near-maximal effects on APD motility. If this were the case, the magnitude of the individual effects of CCK-8 and GLP-1 given alone might have been too great to allow a demonstration of a synergistic interaction when the two peptides were given concurrently. CCK-8 also stimulated the number of IPPWs slightly more than CCK-8 + GLP-1, suggesting that infusion of GLP-1 may have attenuated the effect of CCK-8. Although this has not been described previously, there is evidence to that effect from other gut hormones (12). For example, concurrent intravenous infusion of glucagon and CCK-8 reduces meal size less than the sum of the effects of glucagon and CCK-8 alone (12).

Some limitations of our study must be recognized. Only male volunteers were included, and, accordingly, our observations may not be applicable to females. As only nine subjects were studied, the possibility of a type 2 error should be considered, although, as stated, the number of subjects recruited was based on power calculations from our previous studies (7, 26).

In summary, our study indicates that, at the doses evaluated, CCK-8 and GLP-1 have discrepant effects on appetite, energy intake, and APD motility. Infusion of CCK-8, but not GLP-1, decreased perceptions of appetite and energy intake. Furthermore, although both CCK-8 and GLP-1 had significant effects on antral and duodenal PWs, the effect of the combination of CCK-8 + GLP-1 did not exceed the sum of the effects of CCK-8 and GLP-1 alone, providing no evidence of synergism. Finally, infusion of CCK-8, but not GLP-1, suppressed energy intake and stimulated phasic and tonic pyloric pressures. In view of this, and other work (45), the relationship between energy intake and pyloric motility requires further exploration.

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