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Cholecystokinin-33, but not cholecystokinin-8 shows gastrointestinal site specificity in regulating feeding behaviors in male rats

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Abstract

Two separate experiments were performed to localize the gastrointestinal sites of action regulating meal size (MS), intermeal interval (IMI) length and satiety ratio (SR, IMI/MS) by cholecystokinin (CCK) 8 and 33. Experiment 1: CCK-8 (0, 0.05, 0.15, 0.25 nmol/kg) was infused in the celiac artery (CA, supplies stomach and upper duodenum) or the cranial mesenteric artery (CMA, supplies small and part of the large intestine) prior to the onset of the dark cycle in free feeding, male Sprague Dawley rats and MS (normal rat chow), IMI and SR were recorded. Experiment 2: CCK-33 (0, 0.05, 0.15, 0.25 nmol/kg) were infused in the CA or the CMA, under the same experimental conditions above, and MS, IMI and SR were recorded. Experiment 1 found that CCK-8 reduces MS, prolongs the IMI and increases the SR at sites supplied by both arteries. Experiment 2 found that CCK-33 reduces MS and increases the SR at sites supplied by the CMA. We conclude that in male rats the feeding behaviors evoked by CCK-33, but not CCK-8, are regulated at specific gastrointestinal sites of action.

Keywords

Cholecystokinin; Meal Size; Intermeal Interval; Celiac Artery; Cranial Mesenteric Artery

Introduction

Cholecystokinin (CCK) is a gut-brain peptide secreted by the I cells of the small intestine and evokes physiological responses (Sayegh, 2013) such as reduction of food intake (Gibbs et al., 1973) and contraction of the gallbladder (Ivy, 1928). Depending on the number of amino acids in the peptide chain there are multiple molecular forms of CCK. Cholecystokinin-8 is the shortest active form of CCK, CCK-33 is the originally discovered form of the peptide (Mutt and Jorpes, 1971) and CCK-58 is the only endocrine form of CCK in the rat (Reeve et al., 2003).

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Localizing the sites of action that regulate the various feeding responses evoked by the different forms of CCK is essential to determine their potential anti-obesity role. Recently, we have shown that the area supplied by the celiac artery (CA, stomach and small intestine) regulates reduction of meal size (MS) by CCK-58 and the area supplied by the cranial mesenteric artery (CMA, supplies small and large intestine) regulates prolongation of the intermeal interval (IMI) by this peptide (IMI) (Sayegh et al., 2015; Washington et al., 2016b). The goal of the current work is to localize the gastrointestinal sites of action that regulate the various feeding responses evoked by two forms of CCK, CCK-8 and CCK-33.

One study in the literature utilized an intra-arterial catheterization technique to determine the sites of action for CCK-8. Cox has shown that the area supplied by a branch of the CA contains site(s) of action regulating reduction of cumulative food intake by CCK-8 in rats (Cox, 1998). Although carefully designed, this study suffered the following major deficiencies. (A) It measured cumulative food intake. (B) It measured food intake in food deprived rats. (C) It used a hedonic 30% sucrose test-meal.

Cholecystokinin is a short-term satiety peptide, which regulates meal size and IMI length (Sayegh, 2013), not cumulative food intake. Therefore, a site of action regulating reduction of cumulative food intake by CCK-8 simply does not exist. In addition, it is plausible that MS and IMI length are regulated at different gastrointestinal sites, similar to what has been shown with CCK-58 (Sayegh et al., 2015; Washington et al., 2016b). The first experiment of the current work was designed to determine the site of action regulating MS, IMI length, satiety ratio ($SR=IMI/MS$, or amount of food consumed per a unit of time), size of each meal, duration of individual meal, latency to the first meal, number of meals and total food intake by CCK-8. Such behaviors are necessary to determine the process by which CCK-8 reduces food intake and potentially treats obesity.

Furthermore, Cox measured 30% sucrose intake by CCK-8 in food-deprived rats (Cox, 1998). However, it has been shown that sucrose and fasting stimulate the release of other satiety peptides such as glucagon like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY), which alter the satiety effects evoked by CCK (Geraedts et al., 2012; Imamura et al., 1998; Koop et al., 1987; Ma et al., 2009; McMinn et al., 2000; Peitl et al., 2010; Pritchett and Hajnal, 2012; Wu et al., 2012). Our first experiment here measured normal chow intake by CCK-8 in free feeding male adult rats. Such design is essential to determine the natural feeding responses evoked by CCK-8 and the sites of action that regulate them.

The second experiment in the current study was designed to determine the sites of action regulating MS, IMI, SR, size of each meal, duration of individual meal, latency to the first meal, number of meals and total food intake by CCK-33. This is the first study in the literature to investigate such sites by CCK-33 in male rats. While CCK-8 did not have a specific site to regulate the previous feeding behaviors, CCK-33 reduced meal size and increased the satiety ratio at sites supplied by the cranial mesenteric artery.

Materials and Methods

The Tuskegee University Animal Care and Use Committee approved the animal protocols for the two experiments. Adult male Sprague Dawley rats weighing between 400 and 450 g (n=12 rats divided into two groups, with six rats in each group, CA and CMA respectively) were used for both experiments. The rats were housed in the cages of the BioDAQ E2 system in a controlled environment (12 h dark/12 h light cycle – lights off at 1800 h, 21.5°C), with *ad lib* water and pellet rodent chow (Teklad, WI).

Vascular Catheterization

The catheterization procedures have been described in detail in our recent work (Sayegh et al., 2015; Washington et al., 2014; Washington et al., 2016a; Washington et al., 2016b; Williams et al., 2016). All catheters (Micro-Renathane *Braintree Scientific* R-ITC-SP 9.5) were 9.5" in length. The size of the portion of the catheter threaded into each of the vessels was MRE-010 .010 O.D. × .005 I.D. (.25 OD × .12 ID mm), and the size of the remaining part was MRE-033 .033 O.D. × .014 I.D. (.84 OD × .36 ID mm).

All catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5×/18B, 1×250), general anesthesia (1 ml/kg body weight intramuscularly from a mixture of 5.0 ml of Ketaset [10 mg/kg], 2.5 ml of Rompun® [xylazine 2 mg/kg], 1.0 ml of acepromazine maleate® [1mg/kg] and 1.5 ml of saline prepared in our laboratory) and a ventral midline celiotomy incision. The abdominal wall was prepared for surgery by clipping and cleaning with three alternating betadine solution and alcohol swabs. The ventral midline celiotomy incision was performed following the absence of a pedal reflex, which denotes the beginning of the surgical stage in the animal.

Catheterization of the CA

The CA, a branch of the abdominal aorta, was exposed. A temporary ligation was placed at the base of the artery to prevent bleeding. The CA was punctured just below the base (1–2 mm) with a sterile 30-gauge needle, and the catheter was threaded into the artery. The catheter was fixed in place using cyanoacrylate glue (super glue) at the point of entry. The temporary ligation was removed to allow blood flow through the artery, and the catheter was threaded out of the abdominal cavity subcutaneously between the two scapulae and secured dorsally on the skin with sutures and cyanoacrylate glue.

Catheterization of the CMA

The CMA is a branch of the aorta located caudal (inferior) to the CA. It was exposed and ligated at the base, and the catheter was placed and secured similar to the method described above for the CA.

The muscles of the abdominal wall were closed using a polydioxanone II (4-0) absorbable suture material in a simple continuous pattern, and the skin was closed using surgical staples. Postoperative care included Metacam® (Meloxicam® [1.1 mg/kg]) subcutaneously for pain control and Baytril® (Enrofloxacin® [0.05 ml]) intramuscularly as an anti-bacterial medication. The drugs were given for the initial five days immediately following the

surgeries, and all rats were allowed two weeks of recovery time before we started measuring their food intake. The criteria for complete recovery following surgery included the absence of clinical signs (e.g., pain, red-line around the eye (lack of porphyrin secretion), cold extremities and lethargy) and the return of food intake (rat chow) to baseline levels before the surgery. All catheters were flushed twice daily (0900 h and 1700 h) with heparinized saline (0.3 mL).

Verification of the Surgeries

Confirmation of the catheter site and specificity at the time of catheterization was performed using two methods. First, during surgery, we injected sterile saline in the catheter (0.5 mL) in the catheters. In the CA, the stomach and upper duodenum became pale following the saline injection, whereas the small and part of the large intestine became pale following infusion of saline in the CMA. Second, also during the surgery, the catheters were infused with methylene blue (0.5 ml). The blue dye labeled the stomach and upper duodenum if it was injected in the CA, whereas it labeled the small and part of the large intestine if it was injected in the CMA. Third, at the end of the experiment, the rats were sacrificed with an overdose of pentobarbital, and the catheters were infused with latex, which also revealed the same labeling above in the CA and the CMA.

The BioDAQ Food and Water Intake Monitor

The BioDAQ E2 Food and Water Intake system reports food intake data as bouts, the smallest unit of episodic intake. The unique design allows for minimal food spillage and hoarding of pelleted diets. A tray at the bottom of the hopper captures crumbs to ensure more accurate measurement of food intake. The computerized data stream associated with each bout includes a time and date stamp marking the initiation of intake activity, the period of the activity, and the weight of food or water consumed. The period of a bout varies and is defined by the animal's activity.

Habituation and Formation of Baseline Food Intake

All 12 rats were allowed two weeks of recovery time following the surgery. Immediately after recovery, the rats were habituated to the laboratory environment and the experimental design daily for two extra weeks. At 1745 h, 15 min before lights off, each rat was weighed, handled for a few minutes and received a 0.3-ml infusion of heparinized saline in the CA or the CMA. At 1800 h, lights were off, gates were opened and all rats received normal rat chow and tap water at all times. Every bout of food was recorded automatically, and the data for each rat were collected every 24 hours. The data were analyzed to determine the first meal size and IMI. These data formed the baseline of intake for each of the 12 rats. Based on the manufacture's recommendation and data in our field (e.g. (Chen et al., 2014; Farley et al., 2003; Kowalski et al., 2004)), the criteria for a meal included consumption of 0.2 gm or more, and the criteria for IMI included resting, grooming or showing any behavior other than eating for 15 min or more. The values of this baseline for each rat were compared later with the values of the saline treatment during the experiment. If this baseline data were not equal, or within two standard deviations difference, from the saline value during the experiment for each individual animal then the data were excluded from the analysis. All

data points from all 12 animals were included in the analysis since all data points fell within two standard deviations.

Experiment 1

Food Intake for CCK-8—After we established the baseline intake for all animals, infusion of the peptide or saline vehicle started. At 1745 h on Mondays, Wednesdays and Fridays all rats received a heparinized saline infusion in the CA or the CMA according to Table 1. At 1745 h on Tuesdays, Thursdays and Saturdays, the rats received infusions of CCK-8 (0.05, 0.15 and 0.25 nmol/kg) or saline vehicle in the CA or the CMA, again according to the schedule outlined in Table 1. All doses of CCK-8 were randomly assigned and rotated between all animals until every rat received all doses as well as saline (Table 1).

Immediately following the infusion(s), at 1800 h, normal rat chow and water were presented and remained in the cages at all times. Sundays were reserved for the maintenance of the cages and the BioDAQ system, but the catheters were flushed with 0.3 ml of the heparinized saline solution every day. The data collection started immediately following the presentation of chow and was analyzed for each rat every 24 hours to determine the first MS and IMI.

Experiment 2

Food Intake for CCK-33—Following the completion of Experiment 1, all 12 rats continued to be given saline infusion daily at 1745 h in CA and the CMA for one week before the beginning of Experiment 2. This practice was done to insure returning of the rats their baseline food intake. On the experiment day, and similar to the design of Experiment 1 above, the rats received CCK-33 (0.05, 0.15 and 0.25 nmol/kg) or saline vehicle in the CA or the CMA, again according to the schedule outlined in Table 1 and data were collected automatically by the BioDAQ system.

Statistical Analysis

In both experiments the data for the 12 rats met the stability criterion from the data of the saline test days. Therefore, all data points for all 12 rats for both experiments were included in the analysis. The data from each experiment was analyzed separately using a mixed-model two-way analysis of variance (route $\times$ dose) and repetition on dose with a Bonferroni *t*-test for comparisons using a SYSTAT13 computer software for statistical analysis. Results were considered significant if *p* was equal to or less than 0.05. Data are displayed as the mean $\pm$ standard error of the mean (SEM).

Results

Experiment 1

CCK-8

Meal Size: ANOVA revealed a main effect of dose ($F_{3,40}=5.33$, $p=0.003$ $\eta^2=0.26$), but no main effect of route ($F_{1,40}=0.61$, $p=0.003$ $\eta^2=0.01$) or an interaction between route and dose ($F_{3,40}=0.20$, $p=0.88$ $\eta^2=0.01$). Meal size was reduced by CCK-8 (0.05, 0.15, 0.25 nmol/kg) relative saline vehicle ($p=0.04$, 0.01, 0.006 respectively) (Figure 1).

IMI: ANOVA revealed a main effect of dose ($F_{3,40}=3.35$, $p=0.02$ $\eta^2=0.19$) but no main effect of route ($F_{1,40}=0.01$, $p=0.929$ $\eta^2=1.52e^{-4}$) or an interaction between dose and route ($F_{3,40}=0.81$, $p=0.49$ $\eta^2=0.04$). The IMI was prolonged by CCK-8 0.15 and 0.25 nmol/kg relative to saline vehicle ($p=0.04$ and 0.04) but not 0.05 nmol/kg ($p=0.11$) (Figure 1).

SR: ANOVA revealed a main effect of dose ($F_{3,40}=5.86$, $p=0.001$ $\eta^2=0.30$) but no main effect of route ($F_{1,40}=0.24$, $p=0.62$ $\eta^2=4e^{-3}$) or an interaction between dose and route ($F_{3,40}=0.19$, $p=0.9$ $\eta^2=0.01$). The SR was increased CCK-8 (0.05, 0.15, and 0.25 nmol/kg) relative to saline vehicle ($p=0.02$, 0.01 , and 0.002 respectively) (Figure 1).

Latency to the First Meal: ANOVA revealed a main effect of route and dose ($F_{3,40}=3.29$, $p=0.03$ $\eta^2=0.16$) but no effect of dose ($F_{3,40}=2.51$, $p=0.07$ $\eta^2=0.13$) or route ($F_{1,40}=0.91$, $p=0.34$ $\eta^2=0.02$). The latency to the first meal was prolonged by CCK-8 0.05 nmol/kg relative to saline vehicle and by the CMA more so than the CA route ($p=0.02$) (Figure 2) but not CCK-8 0.15 and 0.25 nmol/kg ($p=0.99$ and 0.69 respectively).

Duration of the first meal: ANOVA revealed an interaction between route and dose ($F_{3,40}=3.94$, $p=0.01$ $\eta^2=0.21$) but no main effect of dose ($F_{3,40}=1.61$, $p=0.2$ $\eta^2=0.08$) or route ($F_{1,40}=0.67$, $p=0.41$ $\eta^2=0.01$). The duration of the first meal was shortened by CCK-8 0.05 and 0.25 nmol/kg relative to saline vehicle and by the CA more so than the CMA route ($p=0.01$ and 0.03 respectively) (Figure 2).

Total Intake: ANOVA revealed a main effect of dose ($F_{3,40}=12.84$, $p=0.001$ $\eta^2=0.44$) but no main effect of route ($F_{1,40}=0.35$, $p=0.55$ $\eta^2=4e^{-3}$) or an interaction between route and dose ($F_{3,40}=2.41$, $p=0.08$ $\eta^2=0.08$). Total intake was reduced by CCK-8 (0.05, 0.15 and 0.25 nmol/kg) relative to saline vehicle ($p=0.001$) (Figure 2).

Number of Meals: ANOVA revealed a main effect of dose ($F_{3,40}=5.95$, $p=0.001$ $\eta^2=0.28$) but no effect of route ($F_{1,40}=1.52$, $p=0.22$ $\eta^2=0.02$) or an interaction between route and dose ($F_{3,40}=1.32$, $p=0.28$ $\eta^2=0.06$). The number of meals was reduced by CCK-8 (0.05, 0.15 and 0.25 nmol/kg) relative to saline vehicle ($p=0.015$, 0.007 and 0.003 respectively) (Figure 2).

Experiment 2

CCK-33

Meal Size: ANOVA revealed a main effect of dose ($F_{3,40}=9.57$, $p=0.001$ $\eta^2=0.36$) and route ($F_{1,40}=7.11$, $p=0.01$ $\eta^2=0.08$) but no interaction between route and dose ($F_{3,40}=1.09$, $p=0.36$ $\eta^2=0.04$). Meal Size was reduced by CCK-33 0.05 nmol/kg ($p=0.03$) given in the CA and by 0.05, 0.15 and 0.25 nmol/kg given in the CMA relative to saline vehicle ($p=0.006$, 0.001 , 0.001 respectively). CCK-33 0.25 nmol/kg produced greater reduction than 0.05 nmol/kg given in the CMA ($p=0.02$) (Figure 3).

IMI: ANOVA revealed no main effect of route ($F_{1,40}=0.27$, $p=0.6$ $\eta^2=0.01$) or dose ($F_{3,40}=1.97$, $p=0.13$ $\eta^2=0.12$) or an interaction between route and dose ($F_{3,40}=0.19$, $p=0.89$ $\eta^2=0.01$). CCK-33 (0.05, 0.15 and 0.25 nmol/kg) failed to prolong the intermeal interval relative to saline vehicle ($p=0.99$, 0.95 , and 0.92 respectively) (Figure 3).

SR: ANOVA revealed a main effect of dose ($F_{3, 40}=7.79$, $p=0.001$ $\eta^2=0.32$) and route ($F_{1, 40}=4.93$, $p=0.03$ $\eta^2=0.06$) but no interaction between route and dose ($F_{3, 40}=1.56$, $p=0.21$ $\eta^2=0.06$). The SR was increased by CCK-33 (0.05, 0.15 and 0.25 nmol/kg) given in the CMA relative to saline vehicle ($p=0.04$, 0.005 and 0.001 respectively). CCK-33 0.25 nmol/kg produced greater increase than CCK-33 0.05 nmol/kg ($p=0.014$) (Figure 3).

Latency to the First Meal: ANOVA revealed no main effect of route ($F_{1, 40}=1.26$, $p=0.26$ $\eta^2=0.02$) or dose ($F_{3, 40}=0.48$, $p=0.69$ $\eta^2=0.02$) or an interaction between route and dose ($F_{3, 40}=2.13$, $p=0.11$ $\eta^2=0.13$). CCK-33 (0.05, 0.15 and 0.25 nmol/kg) failed to prolong the latency to the first meal relative to saline vehicle ($p=1.00$) (Figure 4).

Duration of the first meal: ANOVA revealed no main effect of route ($F_{1, 40}=6e^{-5}$, $p=0.99$ $\eta^2=1.46e^{-7}$) or dose ($F_{3, 40}=0.07$, $p=0.97$ $\eta^2=5e^{-3}$) or an interaction between route and dose ($F_{3, 40}=0.42$, $p=0.73$ $\eta^2=0.03$) (Figure 4). CCK-33 (0.05, 0.15 and 0.25 nmol/kg) failed to shorten the duration of the first meal relative to saline vehicle ($p=1.00$).

Total Intake: ANOVA revealed a main effect of dose ($F_{3, 40}=11.49$, $p=0.001$ $\eta^2=0.43$) but no main effect of route ($F_{1, 40}=1.80$, $p=0.18$ $\eta^2=0.02$) or an interaction between route and dose ($F_{3, 40}=1.14$, $p=0.34$ $\eta^2=0.04$). Total intake was reduced by CCK-33 0.15 and 0.25 nmol/kg relative to saline vehicle ($p=0.001$) (Figure 4). CCK-33 0.05 nmol/kg failed to reduce total intake relative to saline vehicle ($p=0.07$).

Meal Number: ANOVA revealed a main effect of dose ($F_{3, 40}=5.39$, $p=0.001$ $\eta^2=0.27$). ANOVA revealed no main effect of route ($F_{1, 40}=0.66$, $p=0.42$ $\eta^2=0.01$) or an interaction between route and dose ($F_{3, 40}=0.41$, $p=0.74$ $\eta^2=0.02$). The number of meals was reduced by CCK-33 0.15 and 0.25 nmol/kg relative to saline vehicle ($p=0.02$ and 0.008). CCK-33 0.05 nmol/kg failed to reduce the number of meals relative to saline vehicle ($p=1.000$) (Figure 4).

Discussion

The first experiment in the current study found no specific site of action regulating meal size reduction, as well as seven other feeding behaviors, evoked by CCK-8. The wall of the entire gastrointestinal tract contains such sites. On the other hand, the second experiment in this work found that the area supplied by the cranial mesenteric artery, small and part of the large intestine, contain sites of action regulating reduction of meal size by CCK-33.

Determining the sites of action that regulate reduction of meal size and prolongation of the IMI by CCK-8 and CCK-33 is essential because the two peptides evoke different feeding responses. For example, intraperitoneal (i.p) injection of CCK-8 decreases meal size, whereas i.p injection of CCK-33 prolongs the IMI (Hsiao and Wang, 1983; Washington et al., 2011). Therefore, the sites of action that regulate the various feeding behaviors evoked by the two peptides require investigation. Two separate experiments in this study addressed this issue.

Utilizing a similar experimental design to the one used here e.g. intra-arterial catheterization of the arteries of the gut, free feeding male rats, measuring eight feeding behaviors and

measuring normal rat chow we have shown that the gastrointestinal tract contains sites of action controlling MS and IMI length by CCK-58 (Sayegh et al., 2015; Washington et al., 2016b), gastrin releasing peptide (Washington et al., 2014; Washington et al., 2016a) and glucagon like peptide-1 (Williams et al., 2016). The current study found that in free feeding male rats consuming normal chow the CA and the CMA supply sites of action regulating meal size reduction by CCK-8, whereas only the CMA supplies gastrointestinal sites of action regulating meal size reduction by CCK-33.

The results of the two experiments can be explained by at least two main possibilities, the wide distribution of CCKAR in the gut and the different receptor state of the receptor. We have shown that CCKAR, which mediates reduction of food intake by CCK (Corwin et al., 1991), is heavily expressed in the duodenum and jejunum and to a less extent in the stomach, ileum, colon and rectum (Lateef et al., 2012). Such wide distribution may explain the spreading of the sites of action that regulate reduction of meal size by CCK-8 along the gastrointestinal tract. These sites are supplied by the CA and the CMA, small and large intestine. Furthermore, it has been shown that CCK-8 reduces food intake in rats by activating the low affinity CCKAR (Weatherford et al., 1993). It is plausible that this affinity state of the CCKAR also exists along the gastrointestinal tract, again the area supplied by the CA and the CMA, where CCK-8 activated and evoked its feeding responses as shown in the first experiment of this study.

The wide distribution of the CCKAR along the gut wall may also explain the long lasting reduction in meal size and prolongation of the IMI by CCK-8. Compared to other routes, e.g. i.p, giving CCK by the intra-arterial route insured reaching of CCK-8 to all possible locations in the gut, supplied by both CA and CMA, and activating the highest possible number of receptors to reduce meal size and prolong the intermeal interval. In other routes, most of the peptide is degraded by various proteases in the peritoneal fluid, the endothelial cells and the blood (Gores et al., 1989; Gores et al., 1986a; Gores et al., 1986b; Keire et al., 2002).

Now, unlike CCK-8, where there was no specific site of action in the gut that regulates meal size or intermeal interval, reduction of meal size by CCK-33 was regulated at sites supplied by the cranial mesenteric artery. This may indicate that CCK-33 activates a specific receptor state located in the small and part of the large intestine rather than along the whole gastrointestinal tract. In addition, CCK-33 maybe activating a different set of CCKAR that is not located in the upper gut, or areas supplied by the celiac artery. These two possibilities, however, require further investigation.

Finally, some of the responses evoked by both peptides did not reach statistical significance. This can be explained by the relatively small number of rats that we tested in these experiments, six rats per route. Localizing the precise gastrointestinal sites of action regulating the feeding responses by any gut peptide requires testing smaller doses of the peptide, catheterization of the branches of the CA and the CMA and increasing the number of animals per group.

Two separate experiments in this study found that Cholecystokinin-33, but not cholecystokinin-8 shows gastrointestinal site specificity in regulating feeding behaviors in male rats.

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Highlights

- Cholecystokinin (CCK) -8 infused in the Celiac Artery (CA) reduced meal size (MS), prolonged the intermeal interval (IMI) and increased satiety ratio (SR)
- CCK-8 infused in the Cranial Mesenteric Artery (CMA) reduced MS, prolonged the IMI and increased the SR
- CCK-33 infused in the CMA reduced MS and increased SR
- CCK-33, but not CCK-8, is regulated by specific sites in the gut

Figure 1. Effect of cholecystokinin-8 infused in the celiac artery and the cranial mesenteric artery on meal size, intermeal interval length and satiety ratio

Cholecystokinin-8 (CCK-8, 0, 0.05, 0.15, and 0.25 nmol/kg) was infused in the celiac artery (CA, supplies the stomach and the upper duodenum) or the cranial mesenteric artery (CMA, supplies the small and large intestines) prior to the onset of the dark cycle in free feeding rats. First meal size (MS, normal rat chow), intermeal interval (IMI, time between first and second meals in min) length and satiety ratio (IMI/MS) were determined. CCK-8 0.05, 0.15 and 0.25 nmol/kg reduced MS, prolonged the IMI and increased satiety ratio relative to saline control. (* denotes significance, $p < 0.05$).

Figure 2. Effect of cholecystokinin-8 infused in the celiac artery and the cranial mesenteric artery on various feeding behaviors

Cholecystokinin-8 (CCK-8, 0, 0.05, 0.15, and 0.25 nmol/kg) was infused in the celiac artery (CA, supplies the stomach and the upper duodenum) or the cranial mesenteric artery (CMA, supplies the small and large intestines) prior to the onset of the dark cycle in free feeding rats and the latency to first meal, duration of the first meal, total intake and number of meals were measured.

Cholecystokinin-8 given by both routes increased the latency to the first meal relative to saline vehicle (* $p < 0.05$), and more so in the CMA compared to the CA ($\dagger p < 0.05$). In the CA route CCK-8 0.05 and 0.25 nmol/kg decreased the duration of the first meal relative saline vehicle. CCK-8 reduced total intake and the number of meals relative to saline vehicle (* $p < 0.05$).

Figure 3. Effect of cholecystokinin-33 infused in the celiac artery and the cranial mesenteric artery on meal size, intermeal interval length and satiety ratio

Cholecystokinin-33 (CCK-33, 0, 0.05, 0.15, and 0.25 nmol/kg) was infused in the celiac artery (CA, supplies the stomach and the upper duodenum) or the cranial mesenteric artery (CMA, supplies the small and large intestines) prior to the onset of the dark cycle in free feeding rats and first meal size (MS, normal rat chow), intermeal interval (IMI, time between first and second meals in min) length and satiety ratio (IMI/MS) were determined. Cholecystokinin-33 0.05 nmol/kg given in the CA reduced meal size relative to saline (* $p < 0.05$). In the CMA all doses of CCK-33 reduced meal size and increased the SR relative

to saline (* $p < 0.05$) and more so by 0.25 nmol/kg dose compared to 0.05 nmol/kg dose (** $p < 0.05$).

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Figure 4. Effect of cholecystokinin-33 infused in the celiac artery and the cranial mesenteric artery on various feeding behaviors

Cholecystokinin-33 (CCK-33, 0, 0.05, 0.15, and 0.25 nmol/kg) was infused in the celiac artery (CA, supplies the stomach and the upper duodenum) or the cranial mesenteric artery (CMA, supplies the small and large intestines) prior to the onset of the dark cycle in free feeding rats and latency to first meal, duration of the first meal, total intake and number of meals were measured. Cholecystokinin-33 decreased total food intake and number of meals relative to saline control ($p < 0.05$).

Table 1

Experimental Design

Animal No.	Route	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Monday	Tuesday
1	CA	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25
2	CA	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline
3	CA	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05
4	CA	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15
5	CA	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25
6	CA	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline
7	CMA	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05
8	CMA	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15
9	CMA	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25
10	CMA	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline
11	CMA	Hep. Saline	CCK-8 0.15	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05
12	CMA	Hep. Saline	CCK-8 0.25	Hep. Saline	Saline	Hep. Saline	CCK-8 0.05	Hep. Saline	CCK-8 0.15
Animal No.	Route	Wednesday	Thursday	Friday	Saturday	Monday	Tuesday	Wednesday	Thursday
1	CA	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25
2	CA	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline
3	CA	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05
4	CA	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15
5	CA	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25
6	CA	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline
7	CMA	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05
8	CMA	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15
9	CMA	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25
10	CMA	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline
11	CMA	Hep. Saline	CCK-33 0.15	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05
12	CMA	Hep. Saline	CCK-33 0.25	Hep. Saline	Saline	Hep. Saline	CCK-33 0.05	Hep. Saline	CCK-33 0.15

A total of 12 rats were utilized for two separate experiments. The animals were divided into two groups (n=6 rats per group). Animals in the first group (1 through 6) were equipped with catheters in the celiac artery (CA), whereas in the second group (7 through 12) the animals were equipped with catheters in the cranial mesenteric artery (CMA). In Experiment 1, on Mondays, Wednesdays and Fridays all

rats received heparinized saline at 1745 h and food gates were opened at 1800 h. On Tuesdays and Saturdays the rats received saline or CCK-8 (0.05, 0.15 and 0.25 nmol/kg) according to their scheduled days in the CA or the CMA. Sunday was reserved for maintenance of the cages. The feeding responses were recorded automatically by the BioDAQ system. One week following the end of experiment 1, Experiment 2 started. Each of the animals received saline or CCK-33 (0.05, 0.15 and 0.25 nmol/kg) according to the design (times and days) above and the same feeding responses were recorded by the same system. The data of the two experiments were analyzed separately by a two way ANOVA and Bonferroni *t*-test for multiple comparisons.