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Celiac and the cranial mesenteric arteries supply gastrointestinal sites that regulate meal size and intermeal interval length via cholecystokinin-58 in male rats

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ABSTRACT

The site(s) of action that control meal size and intermeal interval (IMI) length by cholecystokinin-58 (CCK-58), the only detectable endocrine form of CCK in the rat, are not known. To test the hypothesis that the gastrointestinal tract may contain such sites, we infused low doses of CCK-58 (0.01, 0.05, 0.15 and 0.25 nmol/kg) into the celiac artery (CA, supplying stomach and upper duodenum), the cranial mesenteric artery (CMA, supplying small and most of the large intestines), the femoral artery (FA, control) and the portal vein (PV, draining the gastrointestinal tract) prior to the onset of the dark cycle in freely fed male rats. We measured the first meal size (chow), second meal size, IMI and satiety ratio (SR, IMI/meal size). We found that (1) all doses of CCK-58 given in the CA and the highest dose given in the CMA reduced the first meal size, (2) all doses of CCK-58 given in the CA reduced the second meal size, (3) a CCK-58 dose of 0.15 nmol/kg given in the CA and 0.15 and 0.25 nmol/kg given in the CMA prolonged the IMI, (4) CCK-58 (0.05, 0.15, 0.25 nmol/kg) given in the CA and 0.25 nmol/kg given in the CMA increased the SR, and (5) CCK-58 given in the FA and PV had no effect on the meal size or intermeal interval. These results support our hypothesis that the gastrointestinal tract contains sites of action that regulate meal size and IMI length via CCK-58. The stomach and upper duodenum may contain sites regulating meal size, whereas the small intestine and part of the large intestine may contain sites regulating the IMI.

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Introduction

Cholecystokinin (CCK) is a gut peptide secreted by the I cells of the small intestine (Buffa et al., 1976; Polak et al., 1975). It evokes physiological responses, such as gallbladder contraction (Ivy and Oldberg, 1928), increased pancreatic secretions (Harper and Raper, 1943) and the reduction of food intake (Gibbs et al., 1973). Based on the number of amino acids in the peptide chain, there are three common molecular forms of CCK, CCK-8, CCK-33 and CCK-58. These peptides activate two G-protein coupled receptors, CCK_1 and CCK_2, which are distributed both peripherally and centrally (Sayegh, 2013).

In 2003, Reeve and colleagues reported that CCK-58 is the only detectable endocrine form of CCK in rats (Reeve et al., 2003). This finding was extended by comparing the effects evoked by CCK-58 with those produced by the other forms of the peptide. Several differences were reported between CCK-8 and CCK-58. For example, at some concentrations, CCK-58 stimulates pancreatic fluid secretion while CCK-8 inhibits it (Yamamoto et al., 2005, 2007), and supraphysiological doses of CCK-58, unlike CCK-8, do not cause pancreatitis in rats (Yamamoto et al., 2007). In addition, CCK-58 increased Fos-like immunoreactivity (Fos-LI), a marker for neuronal activation, in the hindbrain and the submucosal plexus of the gut, while CCK-8 increased it in the hindbrain and both plexuses of the gut, myenteric and submucosal (Cooper et al., 2008; Raboin et al., 2008). Furthermore, CCK-58 and CCK-33 reduce meal size and prolong the intermeal interval (IMI), whereas CCK-8 reduces meal size and does not affect or sometimes shortens the IMI (Glatzle et al., 2008; Goebel-Stengel et al., 2012; Sayegh et al., 2014). Therefore, CCK-58 is an essential tool and the most relevant form for studying the physiology of CCK.

In an elegantly designed series of experiments (Cox, 1998; Cox et al., 1995, 1996) to determine the site of action that controls the reduction of food intake by CCK, Cox et al. reported that the action site controlling a reduction of 30% sucrose intake by CCK-8 in fasted rats resides in the vascular bed of the cranial pancreatico-duodenal artery, the cranial portion of the duodenum. Although important, this finding requires

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re-evaluation for the following reasons. First, new, smaller catheters and techniques that allow more precise injection of the peptide have been developed since the early studies. Second, the site of action that controls the reduction of food intake by CCK-58 remains unknown because the Cox papers used CCK-8. While rat CCK-58 is the only detectable endocrine form of CCK in rats (Reeve et al., 2003), there was little rat CCK-58 available until this peptide was synthesized in 2003. Third, controlling food intake requires evaluating both meal size and IMI (Richter, 1922, 1927), not cumulative food intake as in the Cox studies. Fourth, rats are nocturnal eaters (Richter, 1922, 1927), which means that they mostly consume food during the night time. The current study kept this feeding schedule, rather than using daytime feedings as in Cox's studies. Fifth, measuring chow intake (as done here), the normal daily food consumed by rodents, reflects a more realistic method for evaluating food intake in rats than measuring sucrose intake. Finally, measuring food intake in freely fed rats, as performed in these studies, reveals the accurate effect of a given satiety peptide on food intake better than testing those peptides in food-deprived rats as did Cox.

Here, we measured meal size (normal rat chow) and IMI in freely fed male rats in response to CCK-58 that was infused prior to the onset of the dark cycle in the arteries that supply the portions of the gastrointestinal tract, celiac artery (CA, supplies stomach and upper duodenum) and cranial mesenteric artery (CMA, supplies small and large intestine). We found that the CA may supply most of the areas controlling meal size, while the CMA may supply most areas controlling the IMI length via CCK-58.

Materials and methods

Rat CCK-58 was synthesized in the laboratory of Dr. Reeve as described in the literature (Reeve et al., 2004). The purity of the lyophilized peptide was 87%, and the mass and amino acid composition of synthetic rat CCK-58 were indistinguishable from theoretical values.

The Tuskegee University Animal Care and Use Committee approved the animal protocols for this study according to the guidelines of the PHS and USDA. Adult male Sprague Dawley rats weighing between 400 - 450 g (n = 32 divided into four groups, with eight rats in each group, CA, CMA, FA and PV) 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).

Surgical procedures

The surgical procedure has been described in detail in our recent work (Washington et al., 2014a). Thirty-two rats were catheterized in the CA, CMA, FA and PV. All catheters were 9.5" in length. The size of the portion of the catheter (Micro-Renathane *Braintree Scientific* R-ITC-SP 9.5) threaded into each of the vessels was MRE-010 .010 O.D. × .005 I.D in. (.25 OD × .12 ID mm), and the size of the remaining section was MRE-033 .033 O.D. × .014 I.D.in. (.84 OD × .36 ID mm).

All catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5×/18B, 1x250), general anesthesia and a ventral midline celiotomy incision. All animals were given an anesthesia mixture prepared in our laboratory (1 mg/kg body weight intramuscularly; i.m.). The abdominal wall was prepared for surgery by clipping and cleaning with three alternating swabs of betadine solution and alcohol. A 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 is a branch of the abdominal aorta (Fig. 1). It was exposed, and then, a temporary ligation was placed at the base of the artery to prevent bleeding. The CA was then punctured just below the base

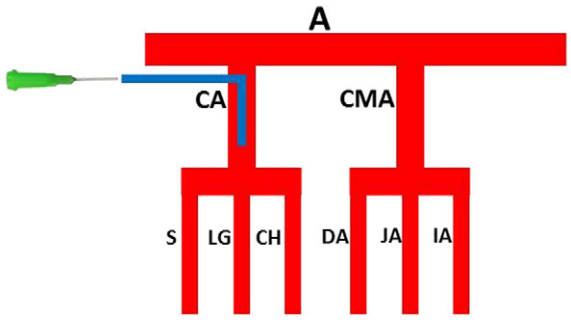

Fig. 1. Arterial supply of the stomach and intestines. The aorta (A) supplies the stomach and intestines by two branches celiac artery (CA) and the cranial mesenteric artery (CMA). The CA, which supplies the stomach and the upper portion of the duodenum, gives three branches, splenic (S), left gastric (LG) and common hepatic (CH). The CMA supplies the small and part of the large intestine by three main arteries, caudal pancreatico-duodenal artery (DA), jejunal arteries (JA) and ileocolic artery (IA). Our catheter was inserted in the CA.

(2-3 mm) with a sterile 30 gauge needle and the catheter was threaded into the artery. The catheter was then fixed in place and sealed using cyanoacrylate glue (super glue) at the point of entry. The temporary ligation was removed to allow blood to flow in the artery. The catheter was then threaded out of the abdominal cavity subcutaneously to appear between the shoulder blades and secured with sutures and cyanoacrylate glue.

Catheterization of the CMA

The CMA is a branch of the aorta located caudally (inferior) to the CA (Fig. 1). It was exposed and ligated at the base. The catheter was placed and secured with methods similar to those described above for the CA.

Catheterization of the FA

The FA was exposed on the medial aspect of the right thigh of the rat, and a microvascular clamp was used to clamp the artery (*Microsurgery Instruments, Inc*. MC6 double clamp 0.9 cm). The artery was then catheterized with similar methods to those described above for the CA.

Catheterization of the portal vein

The portal vein was located and exposed on the ventral aspect of the liver, and catheter placement was performed as 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]) administered subcutaneously for pain control and Baytril® (Enrofloxacin® [0.05 ml]) administered intramuscularly as an anti-inflammatory 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 the food intake experiment. The criteria for complete recovery following surgery included the absence of clinical signs (e.g., pain, red-line around the eye, cold extremities and lethargy) and the return of food intake (rat chow) to baseline levels. 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 (Washington et al., 2014a). First, we injected methylene blue in the catheter (0.5 mL). The blue dye labeled the stomach and upper duodenum in the CA and the