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Duodenal myotomy blocks reduction of meal size and prolongation of intermeal interval by cholecystokinin

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ABSTRACT

We have shown that vagotomy (VGX) attenuates the reduction of meal size (MS) produced by cholecystokinin (CCK) -8 and -33 and that celiaco-mesenteric ganglionectomy (CMGX) attenuates the prolongation of the intermeal interval (IMI) produced by CCK-33. Here, we report the following novel data. First, by determining the distribution of CCK_1 receptor messenger RNA, which mediates reduction of MS and prolongation of IMI by CCK, in seven regions of the gastrointestinal tract in the adult rat we found that the duodenum contains the highest concentration of this receptor in the gut. Second, based on the previous finding we performed a unique surgical technique known as duodenal myotomy (MYO), which severs all the nerves of the gut wall in the duodenum including vagus, splanchnic and enteric nerves. Third, we determined MS and IMI in duodenal MYO rats in responses to endogenous CCK-58 released by the non-nutrient, trypsin inhibitor, camostat and CCK-8 to test the possibility that the duodenum is the site of action for reduction of MS and prolongation of IMI. We found that, similar to the previous work reported by using CCK-8 and MS, duodenal MYO also blocked reduction of MS by camostat. Forth, duodenal MYO blocked prolongation of IMI by camostat. As such, our current results suggest that the duodenum is the gut site that communicates both feeding signals of endogenous CCK, MS and IMI, with the brain through vagal and splanchnic afferents.

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1. Introduction

Cholecystokinin (CCK) is a gut-brain peptide secreted by the endocrine I cells of the small intestine [1–3] and evokes responses consistent with a role in the short-term control of food intake: satiation (reduction of meal size, MS) and satiety (prolongation of intermeal interval, IMI) [4,5]. Such responses by CCK are mediated by the CCK_1 receptors [5–7] and through vagal and splanchnic afferents [7–10].

Reduction of cumulative food intake by CCK-8 and fatty acids, which releases endogenous CCK, was attenuated by selective [9,11–15] or total subdiaphragmatic vagotomy [8]. In addition, we have recently shown that reduction of MS by CCK-8 and -33 was attenuated by total subdiaphragmatic vagotomy [10], whereas prolongation of the IMI by CCK-33 was shortened by removing the celiaco-mesenteric ganglia [10]. These data provide evidence that the feeding responses evoked by exogenous CCK require differential neuronal pathways. However, they do not provide insight into the specific gut area that communicates these feeding signals, MS and IMI, by endogenous CCK to the central feeding control locations in the dorsal vagal complex (DVC) of the hindbrain.

The reasons are as follows: (1) The vagus and splanchnic nerves supply the majority of the gastrointestinal (GI) tract [16–18]; therefore, total subdiaphragmatic vagotomy (VGX) and celiaco-mesenteric ganglionectomy (CMGX)/splanchnictomy (SPX), which we performed in our previous study [10], do not provide knowledge about the specific gut site for these neuronal pathways; (2) The endocrine I cells that are the major source of endogenous CCK in the periphery are also distributed along the whole length of the small intestine [1–3]. As such, all regions of the small intestine that contain these cells (such as the duodenum, the jejunum and the ileum) are possible gut sites for the communication of the feeding signals of CCK with the DVC; (3) Cholecystokinin$_1$ receptor expression, which mediates reduction of food intake by CCK, can be found in the previous regions of the small intestine [19–22]. Thus, any of those regions that contain this receptor are likely gut sites for the communication of the satiation and satiety signals of CCK with the DVC; and finally (4), while all of the previous studies have focused on identifying the pathway by which CCK communicates the satiation signal to the brain [11,23–26], no report has investigated the site of the gut that communicates the satiety signal, or IMI, of CCK with the DVC.

There are multiple studies which have shown that the site of action for CCK-8 and fatty acids, which release endogenous CCK, to evoke reduction of cumulative food intake is the upper gastrointestinal tract, particularly the duodenum [11,23–27]. However, the gut

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site of action for endogenous CCK-58, the only endocrine form of CCK in the rat [28] which is secreted in response to the non-nutrient, trypsin inhibitor camostat [28,29], and evokes reduction of MS and prolongation of the IMI [5,30–32] is yet to be determined.

In the current study, we investigated if the duodenum is the primary site in the gut that communicates these feeding responses, MS and IMI [5,10,33–35] in response to endogenous CCK released by an orogastric gavage of the non-nutrient trypsin inhibitor camostat (200 mg/kg [5,7,36]) and in response to CCK-8 (1 nmol/kg, i.p. [34,35,37]) in duodenally myotomized (MYO) rats. Several previous studies from our laboratory have shown that these doses reduce MS through camostat and CCK-8 and prolong the IMI through camostat [5,10,33–35].

Duodenal MYO (Fig. 1) is a unique and a challenging surgical procedure performed by introducing six adjacent and complete circumferential incisions in the duodenum immediately following the pyloric sphincter. These incisions sever all the layers of the duodenal wall except the mucosa and submucosa. In addition, this procedure severs all of the neuronal elements in the duodenal wall, including the enteric nervous system (ENS) [38], vagal and sympathetic/spinal afferents. The choice of the surgical site for our duodenal MYO was based on the distribution of CCK_1 receptor gene expression in seven regions of the gut. In our study, this receptor, which mediates reduction of food intake by CCK, was mainly distributed in the upper duodenum at the level of and immediately following the pyloric sphincter [11,23–27].

2. Materials and methods

The Tuskegee University Animal Care and Use Committee approved the animal protocols for this study. Adult male Sprague Dawley rats weighing between 250 and 300 g and housed in clear cages, allowing for the complete visualization necessary for behavioral rating, were used in this study. The rats lived in a controlled environment (12-h dark/12-h light cycle; lights off at 1800 h, at 21.5 °C, with *ad libitum* water and pelleted rodent chow, *Teklad, WI*).

To habituate the rats to the laboratory environment and the experimental design, each rat was weighed and handled for 10 min every day at the same time each day and was then given an intraperitoneal (i.p.) injection of saline and gavaged with 3.5 ml double distilled water (ddH_2O), followed by a 10% sucrose solution, for 120 min in addition to chow and water. All injections consisted of a volume of 0.5 ml, and all gavage solutions consisted of a volume of 3.5 ml and were given at 0700 h (at 1 h into the beginning of the light cycle) to allow for the necessary preparation of solutions and needed materials before the experiment.

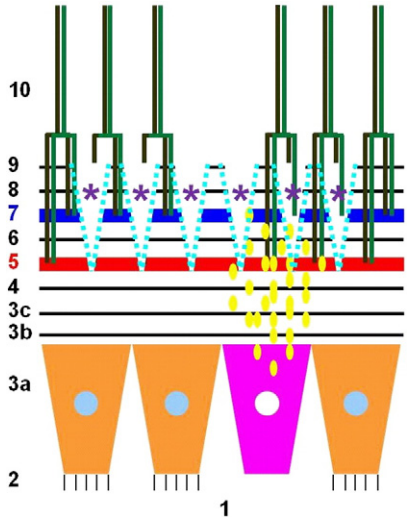

Fig. 1. *Duodenal myotomy*. The gut consists of four layers: the mucosa, submucosa, muscularis and serosa. Starting from the lumen of the gut (1), the mucosa consists of villi (2) -equipped enterocytes (3a, brown) and endocrine cells (purple; e.g., I cells that secrete CCK; yellow shapes), lamina propria (3b) and muscularis mucosa (3c). The submucosa (4) carries a submucosal plexus (5, thick, interrupted red line), which is part of the enteric nervous system (ENS) of the gut. The muscularis consists of an inner circular muscle layer (6), followed by the myenteric plexus (7, thick, interrupted blue line) (the second part of the ENS) and an outer longitudinal muscle layer (8). The fourth layer of the gut wall is the serosa (9). The ENS, myenteric and submucosal plexuses comprise the intrinsic innervation of the gut. The extrinsic innervation of the gut consists of two nerves, the vagus (10, black line; carries a motor and a sensory portion) and the splanchnic (10, green line; carries sympathetic/spinal afferents), which connects to the central nervous system. The purple stars in the middle of the V-shaped light blue dotted lines represent the sites of the myotomy procedure.

2.1. Distribution of CCK_1 receptor

Ten rats were sacrificed with an overdose of sodium pentobarbital (100 mg/kg, i.p.). Approximately 200-mg-long intestinal segments were collected from each rat from the following areas of the intestine: a 1 cm^2 piece of the antrum of the stomach, 0.5 cm aborad from the pyloric sphincter (duodenum), 20 cm aborad from the same point (jejunum), 5–6 cm orad from the cecum (ileum), 1 cm of the cecal body, 5 cm from the cecum aborally (colon) and 1 cm^2 from the terminal part of the gastrointestinal tract (rectum).

Total RNA was isolated using the TRIZOL method (*Invitrogen-Life Technologies, Inc., Carlsbad, CA*) [19,22] according to the manufacturer's protocol, and the total RNA concentration was determined by ultraviolet absorbance at 260 nm (DU640, *Beckman Coulter, Fullerton, CA*). RNA from each sample was assessed for purity by determining the $A_{260/280}$ ratios (ratios of 1.5 to 2 were used), and the integrity of each sample was assessed by samples produced on 2% agarose gels stained with ethidium bromide. All RNA samples were treated with RNase-free DNase (*Ambion, CA*) to remove residual DNA. Samples were selected based on the bright staining of the 18 s and 28 s ribosomal bands, with the latter showing twice the concentration of the former. First-strand cDNA was synthesized from 2 μg total RNA using Reaction Ready™ First Strand cDNA Synthesis (*Super Array Bioscience Corporation, Frederick, MD*).

mRNA levels were measured using RT-PCR in a 25 μl reaction mixture containing 12.5 μl RT^2 Real-Time SYBR/Fluorescein Green PCR master mix, 1 μl first strand cDNA, 1 μl RT^2 validated PCR primer sets for CCK_1 and CCK_2 (*Super Array Bioscience Corporation*) and 10.5 μl PCR-grade water (*Ambion Inc*). Samples were run in 96-well PCR plates (*Bio-Rad, Hercules, CA*) in duplicate, and the results were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPD) housekeeping gene. The amplification protocol was set at 95 °C for 15 min, followed by 40 cycles each at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a melting curve determination between 55 °C and 95 °C to ensure the detection of a single PCR product. A complete account of this protocol has been described in our previous work [19,22].

2.2. Myotomy

Two groups of rats (n = 7 per group) underwent duodenal myotomy (MYO) or sham surgery under general anesthesia through a ventral midline celiotomy incision. The anesthesia mixture (0.01 mg/kg intramuscularly; i.m.) prepared in our laboratory contained 5.0 ml of ketaset (100 mg/ml), 2.5 ml of Rompun® (xylazine 20 mg/ml), 1.0 ml of acepromazine maleate® (10 mg/ml) and 1.5 ml saline. The abdominal wall was prepared surgically by clipping and cleaning with betadine solution and alcohol swabs, and a ventral midline celiotomy was performed following the absence of a pedal reflex.

The stomach and small intestine were exposed, and using micro dissection scissors, six circumferential cuts (3–5 mm apart) were