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The stomach and/or upper duodenum contain sites of action that control meal size and intermeal interval length by exogenous rat gastrin releasing peptide

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ABSTRACT

The site(s) of action that control the reduction of food intake in response to the amphibian skin peptide bombesin (Bn) has been determined to be the area supplied by the celiac artery (CA), i.e., the stomach and the upper duodenum. Here, we investigated the gastrointestinal site(s) of action which controls meal size (MS) (normal rat chow) and intermeal interval length (IMI) by the mammalian homologues of Bn gastrin releasing peptides (GRP-10, GRP-27 and GRP-29, 0.01, 0.05, 0.1, 0.2 and 0.5 nmol/kg) infused in the CA, the cranial mesenteric artery (CMA, supplying the small and large intestine), the femoral artery (FA, control) and the portal vein (PV, draining the gastrointestinal tract, control) in freely fed rats immediately prior to the onset of the dark cycle. We found that (1) GRP-29 (0.05, 0.1, 0.2 and 0.5 nmol/kg) and GRP-27 (0.2 and 0.5 nmol/kg) in the CA and GRP-29 (0.5 nmol/kg) in the CMA reduced the MS relative to saline, (2) GRP-29 (0.1, 0.2 and 0.5 nmol/kg) and GRP-27 (0.2 and 0.5 nmol/kg) in the CA prolonged the IMI, (3) GRP-29 (0.1, 0.2 and 0.5 nmol/kg) in the CA and GRP-29 (0.5 nmol/kg) in the CMA increased the satiety ratio (SR, IMI/MS – the amount of food consumed per a given unit of time) and (4) neither peptide nor route showed any effect on the second MS. These results support an upper gastrointestinal site of action for MS and IMI length by GRP-27 and GRP-29, which is most likely the stomach and/or the duodenum.
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Introduction

Gastrin releasing peptides (GRP) are mammalian homologs of Bombesin (Bn), a 14-amino acid peptide isolated from the skin of the European frog *Bombina bombina*. There are three forms of GRP that are secreted from the enteric neurons: GRP-10, which exists in all species; GRP-27, which exists in all species, except rats; and GRP-29, which exists only in rats [7].

Bombesin and GRP evoke responses, such as hyperthermia, bradycardia, inhibition of gastric emptying and inhibition of food intake. These effects occur via the activation of three G-protein coupled receptors, the GRP-R or BB_1, the NMB-R or BB_2 and the orphan Bn receptor subtype-3 (BRS-3) or BB_3. Bombesin has approximately thirteen times more affinity for the BB_1 receptor than GRP (34 nM for BN vs. 440 nM for GRP) [7].

Although the feeding responses by Bn and GRP, including meal size (MS) and intermeal interval (IMI) length, have been investigated [2,9,12], the site of action for MS and IMI length by the various forms of GRP is still unknown. Based on the following knowledge, we hypothesize that these sites reside in the gastrointestinal tract.

First, GRP is secreted by the enteric neurons of the gut [1]. Second, utilizing *in situ* hybridization [5], Moran and colleagues found that the distribution of GRP is primarily in the stomach, small intestine and colon. Recently, we have shown [7], using a real time polymerase chain reaction test, that the distribution of GRP is primarily in the ileum > cecum > distal duodenum > pyloric sphincter > colon > distal stomach > esophagus > jejunum > proximal duodenum > rectum > proximal stomach medulla of the hindbrain [7]. Third, evoking a reduction of cumulative food intake by Bn and a reduction of MS and IMI length by Bn and GRP requires vagal, capsaicin-sensitive, and splanchnic nerves [8,10,13]; these changes are accompanied by increased Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) in the enteric neurons of the stomach and the duodenum and in food control areas in the hindbrain, including the area postrema (AP), the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV) [4,11]. Fourth, in 1991, Kirkham and colleagues [3] demonstrated that

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infusing Bn (1, 2 and 4 μg/kg) in the artery supplying the stomach and upper duodenum, the celiac artery (CA), reduced food intake more than infusions of the same doses of the peptide in the cranial mesenteric artery (CMA, supplies small and large intestine) and intraperitoneally. Thus, the gastrointestinal tract is a potential area which may contain control sites that regulate MS and IMI length by GRP.

To test this hypothesis, we equipped free feeding undisturbed male Sprague Dawley rats with catheters in the CA, the CMA, the femoral artery (FA) and the portal vein (PV, drains the gastrointestinal tract) and infused them with GRP-10, GRP-27 and GRP-29 (0.01, 0.05, 0.1, 0.2 and 0.5 nmol/kg) immediately before the beginning of the dark cycle. We determined the MS (regular rat chow), the IMI, the satiety ratio (SR, IMI/MS, which denotes the amount of food consumed per a given unit of time) and the second MS using the BioDAQ system. We found that infusions of GRP-27 and GRP-29 in the CA produced the majority of the effects. In conclusion, the stomach and/or the upper duodenum are potential sites of action that regulate MS and IMI length by GRP-27 and GRP-29.

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 420 and 490 g ($n = 24$ divided into four groups, with six 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

Twenty-four animals were catheterized in the CA, CMA, FA and PV (Micro-Renathane *Braintree Scientific* R-ITC-SP 9.5). All catheters 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 O.D. mm × .12 I.D. mm), and the size of the remaining part was MRE-033 .033 O.D. × .014 I.D. (.84 O.D. mm × .36 I.D. mm).

All catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5×/18B, 1 × 250), 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 betadine solution and alcohol swabs three times each, alternating five min at each swab. 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, and a temporary ligation was placed at the base of the artery to prevent bleeding. The CA was then punctured just below the base (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 (Fig. 1)

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

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.

The muscles of the abdominal wall were closed using a polydioxanone II (4-0) absorbable suture material in a simple continuous pattern; 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-inflammatory medication. The drugs were given in the initial five days immediately following the surgeries, and all rats were allowed two weeks of recovery time before performing a 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 (0.3–0.5 mL).

Verification of the surgeries (Fig. 2)

Confirmation of the catheter site and specificity at the time of catheterization was performed in two ways. 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 small and large intestine in the CMA. Second, we injected sterile saline in the catheter (0.5 mL). In the CA, the stomach and upper duodenum became pale following the injection, whereas in the CMA, the small and large intestine became pale. At the end of the experiment the rats were sacrificed with an overdose of pentobarbital and the catheters were infused with latex. The same tissues were labeled as described above.

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 is variable and is defined by the animal's activity.

Habituation and formation of baseline food intake

Following the two weeks of recovery time immediately after surgery, rats were habituated to the laboratory environment and experimental design every day at 1700 h, i.e., 1 h before the lights were turned off; each rat was weighed, handled for a few minutes and received a 0.3–0.5 mL infusion of heparinized saline in the CA, CMA, FA and PV. At 1800 h, the lights were turned off, gates were opened and all of the rats received normal rat chow and tap water at all times (except for the 1 h period between 1700 h and 1800 h of handling and the infusion of heparinized saline). Every bout of food was recorded automatically, and the data were collected every 24 h; these data were analyzed to determine the first MS, IMI, SR and second MS. This process continued for two weeks and formed the baseline of intake for each of the 24 rats. The values of this baseline for each rat were compared with the values of the saline treatment days during the experiment. If these data did not match,