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Intravenous infusion of gastrin-releasing peptide-27 and bombesin in rats reveals differential effects on meal size and intermeal interval length

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Abstract

We have previously shown that the intraperitoneal (i.p) administration of gastrin-releasing peptide-27 (GRP-27) or bombesin (BN) (at 0.21, 0.41 and 1.03 nmol/kg) reduces meal size (MS) and prolongs the intermeal interval (IMI). Here, we hypothesized that the intravenous (i.v) administration of the same doses of GRP-27 and BN will be as effective as the i.p administration in evoking these feeding responses. To test this hypothesis, we administered GRP-27 and BN i.v and measured first MS (10% sucrose), IMI, satiety ratio (SR, IMI/MS) and second MS in overnight food-deprived but not water-deprived male Sprague Dawley rats. We found that (1) only GRP-27 reduced the first MS, (2) BN prolonged the IMI, (3) GRP-27 and BN increased the SR and (4) only BN reduced the size of the second meal. Contrary to our hypothesis, the i.v administration of GRP-27 and BN affected the MS and IMI differently than did the i.p administration. In conclusion, this pharmacological study suggests that the MS and IMI are regulated at different sites.

Keywords

Gastrin-Releasing Peptide; GRP; Bombesin; Meal Size; Intermeal Interval; Gastrointestinal Tract

Introduction

Bombesin (BN) is a 14-amino-acid peptide that was isolated from the skin of the European frog *Bombina bombina*. Homologs of this peptide, which are secreted from the enteric neurons of mammals, include three forms of gastrin-releasing peptide (GRP) and a 10-aminoacid peptide referred to as neuromedin B (NMB). The three forms of GRP include GRP-10, the small form of GRP that exists in all species; GRP-27, the large form of GRP

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that exists in all species except rats; and GRP-29, the large form of GRP that exists only in rats [25].

Bombesin and GRP activate three G protein-coupled receptors to evoke their responses. These receptors are GRP-R or BB₁, NMB-R or BB₂ and an orphan Bn receptor subtype-3 (BRS-3) or BB₃. Gastrin-releasing peptides and BN bind the BB₁ receptor. However, BN has approximately thirteen times more affinity for the BB₁ receptor than does GRP (34 nM for BN vs. 440 nM for GRP). The activation of these receptors by BN and GRP evokes responses such as hyperthermia, bradycardia, inhibition of gastric emptying and inhibition of food intake [25].

The reduction of food intake by BN and GRP has been previously investigated. Intraperitoneal (i.p) injections of BN and GRP reduce the meal size (MS) and prolong the intermeal interval (IMI) length [5, 29, 36]. Furthermore, Rushing and colleagues [24] have shown that the intravenous (i.v) administration of GRP-27 (at 5 and 10 µg/kg) following the end of the first meal to freely feeding, non-disturbed rats prolongs the IMI.

Possible pathways for the previously identified GRP- and BN-induced feeding responses have been evaluated. For example, reduction of the MS and prolongation of the IMI by BN and GRP-29 require vagal, capsaicin-sensitive, and splanchnic nerves [15, 28, 31, 37]. Such responses are accompanied by increased Fos-like immunoreactivity (Fos-LI, a marker for neuronal activation) in the enteric neurons of the stomach and the duodenum [35] and in food control areas in the hindbrain [14-16], including the area postrema (AP), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) [13, 35].

Despite the previous data, two questions remain unanswered. First, what is the site(s) of action that regulates the MS and IMI length by GRP? Second, are these feeding responses regulated by the same site or different sites? To answer these questions, we hypothesized that if the MS and IMI length are regulated by the same site(s), then administering the peptides via i.v will produce similar effects as did the i.p administration, which we previously tested [36] using the same doses and experimental design. On the other hand, if the MS and IMI length are regulated by different sites, then administering GRP-27 and BN via i.v will affect the MS and IMI length differently than did the i.p administration.

To test this hypothesis, we equipped overnight food- but not water-deprived rats with i.v catheters in the femoral vein (FV) and infused them with various doses of GRP and, for comparative purposes, BN. Immediately following the infusion, we measured their food intake (10% sucrose) and rated their behavior every minute to determine the size of the first two meals, the time between them (the IMI), and the satiety ratio (SR; IMI (min)/MS (ml) – defined as the amount of food that is consumed per a given unit of time). The current study suggests that the regulation of the MS and IMI length by GRP-27 and BN occurs at different sites, which we propose to be gastrointestinal sites.

Materials and Methods

Experimental Procedures

The Tuskegee University Institutional Animal Care and Use Committee approved the protocols for all of the experiments (R1108-14-1). Adult male Sprague Dawley rats (n=8, 350-400 g; Harlan, IN, USA) were housed individually in special plastic cages to allow for the visualization of the animals from all sides for complete behavioral analysis during the food intake experiment. The rats were housed in a controlled environment with a 12 hr light/12 hr dark cycle at a temperature of 21°C and given free access to rodent chow pellets and water. To adapt the animals to the experimental protocol, each rat was handled daily for at least 10 min and given a 10% sucrose solution in addition to rat chow and water, and the catheters were flushed with heparinized saline.

Surgical Procedure

Eight animals underwent femoral vein (FV) catheterization using a surgical microscope (*Carl Zeiss OPMI 160, 12.5x/18B, 1x250*), general anesthesia (1 mg/kg body weight, intramuscularly prepared in our laboratory by mixing 5.0 ml Ketaset (100 mg/ml), 2.5 ml Rompun® (xylazine 20 mg/ml), 1.0 ml acepromazine maleate® (10 mg/ml) and 1.5 ml saline) and an incision in the medial aspect of the thigh. Following the absence of the pedal reflex, which denotes the surgical stage of anesthesia and the animal readiness for surgery, the medial surface of the thigh was clipped and surgically prepared alternatively with Betadine solution and alcohol swabs three times for three minutes each time. A 3-cm incision was made in the medial aspect of the right thigh to expose the FV. Following the complete freeing of the vein from the surrounding tissue/fascia, a microvascular clamp (*Microsurgery Instruments, Inc. MC6 double clamp 0.9 cm*) was applied to the vein (*Acland's Practice Manual for Microvascular Surgery by Robert D. Acland, 2008*). The FV was then punctured just below the base with a sterile 30-gauge needle, and the catheter (*Micro-Renathane Braintree Scientific MRE-025 .025 O.D.X.012 I.D.*) was threaded into the vein. The catheter was then fixed into place using a non-absorbable suture material (stay suture, silk, size 6-0, *Ethicon*) and sealed at the point of entry by applying cyanoacrylate glue (super glue) to the catheter and vein, and the clamp and the stay suture were removed to allow blood flow to the vein. The catheter was then threaded out subcutaneously to the back of the head between the shoulder blades (scapula), where it was secured with sutures and super glue. All of the catheters were flushed twice daily with heparinized saline (0.5 ml). The muscles of the thigh region were closed using a polydioxanone II (5-0) absorbable suture material, 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-inflammatory. The drugs were given in the initial three days immediately following the surgeries, and all of the rats were allowed seven days for recovery before any food intake experiment was performed. 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 to baseline levels.

Food Intake

A baseline of food intake for all of the rats was determined by the following procedure. The rats were fasted of food but not water at 1700 h (one hour before lights were off) on the day prior to the experiment. At 0700 h (one hour after lights were on) the following morning, all of the rats received an infusion of saline. Immediately following the infusion, the rats were presented with a 10% sucrose solution. The intake of sucrose was measured every 5 min for a total of 120 min, and the behavioral rating of the feeding was recorded every minute to determine the MS, IMI and SR for the total duration of the test. The baseline was established by two independent examiners who were blinded to all procedures and treatments with an agreement rate that exceeded 98%. This technique has been utilized extensively in our previous work [7, 17, 18, 34, 36, 37] and was based on previous literature by Gibbs and colleagues [1, 4, 6, 9, 11, 12, 30].

The animal feeding behaviors included feeding (licking the sucrose bottle), licking (licking the water bottle), biting, grooming, locomotion (walking around the cage), rearing up (front paws on side of cage while hind paws on the floor of cage), standing (only hind paws touching floor of cage), sniffing and stretching and resting (no movement). A meal was determined by three consecutive resting periods over three consecutive minutes. Following this baseline, the treatments began.

All of the rats were fasted as described above. The next morning, based on our previous dose-response curve [36] the rats were given an infusion of GRP-27 and BN (0.21, 0.41, and 1.03 nmol/kg) or saline vehicle on odd days and only a saline injection on even days. One day was reserved to maintain the cages. All of the treatments were repeated until each animal received each treatment, which insured that each animal served as a control for itself. Immediately following the infusion, the intake and behavioral rating were recorded as described above. Following these ratings, the first and second meals and the time between them were calculated. Finally, the satiety ratio was calculated by dividing the intermeal interval (min) by the size of the first meal (ml).

Statistical Analysis

The saline treatments agreed with the baseline data, which were collected before the experiment started. The saline treatments were averaged and considered the control for both GRP-27 and BN. The data were analyzed by a two-way analysis of variance (ANOVA) with two variables: treatment and dose. Multiple comparisons were performed using the Holm-Sidak test (*SigmaStat for Windows version 3.11*). All of the results are displayed as the mean $\pm$ SEM, and the data were considered significant when $p < 0.05$.

Results

Effect on First Meal Size

There was an effect of the treatment ($F(1, 27) 37.292$ $p < 0.001$), there was no effect of the dose ($F(3, 27) 2.154$ $p = 0.125$) and there was an interaction between the treatment and the dose ($F(3, 27) 6.107$ $p = 0.004$). GRP-27 (0.21, 0.41, 1.03 nmol/kg) reduced the meal size compared to the effect of the saline control ($p = 0.026, 0.012, 0.001$). Bombesin failed to

reduce the first meal size compared to the effect of the saline control ($p=0.097$, 0.798 , 0.375).

Effect on Intermeal Interval

There was an effect of the treatment ($F(1, 27) 66.740$ $p<0.001$) and the dose ($F(3, 27) 37.744$ $p<0.001$), and there was an interaction between the treatment and the dose ($F(3, 27) 12.926$ $p<0.001$). Only GRP-27 (1.03 nmol/kg) prolonged the size of the intermeal interval compared to the effect of the saline control ($p<0.001$). All of the doses of Bombesin (0.21 , 0.41 , 1.03 nmol/kg) prolonged the size of the intermeal interval compared to the effect of the saline control ($p=0.001$, 0.003 , 0.001).

Effect on Satiety Ratio

There was an effect of the treatment ($F(1, 18) 7.552$ $p=0.013$) and of the dose ($F(3, 18) 9.483$ $p<0.001$), but there was no interaction between the treatment and the dose ($F(3, 18) 1.894$ $p=0.167$). GRP-27 (1.03 nmol/kg) increased the satiety ratio compared to the effect of the saline control ($p=0.017$). Bombesin (0.21 , 1.03 nmol/kg) increased the satiety ratio compared to the effect of the saline control ($p=0.012$, 0.001).

Effect on Second Meal

There was no effect of the treatment ($F(1, 18) 0.150$ $p=0.703$) or of the dose ($F(3, 18) 1.170$ $p=0.349$) nor was there an interaction between the treatment and the dose ($F(3, 18) 1.389$ $p=0.278$). GRP-27 (0.21 , 0.41 , 1.03 nmol/kg) failed to reduce the size of the second meal compared to the effect of the saline control ($p=0.059$, 0.631 , 0.836). Only Bombesin (0.21 nmol/kg) reduced the size of the second meal compared to the effect of the saline control ($p=0.009$).

Discussion

The current work tested the hypothesis that the i.v administration of GRP-27 and BN, similar to the i.p administration [36], reduces the MS and prolongs the IMI length. Unlike the i.p administration, however, we found that the i.v administration of only GRP-27 reduces the MS, while that of BN prolongs the IMI. These differential effects by the two routes suggest that the feeding responses by GRP-27 and BN are regulated by different sites, which we propose to be gastrointestinal sites.

The reduction of the MS and the prolonging of the IMI length by GRP and BN, respectively, have been documented prior to this report [5, 10, 19, 29, 30, 33, 36]. Here, we elected to test GRP-27 for two reasons. First, GRP-27 is the large molecular form of GRP, which exists in humans, a species where the study of the control of food intake is important, especially for its role in the fight against obesity. Second, we tested the same doses of GRP-27 in this study as in our previous work [36]. BN was used in this work for comparative purposes to study the pharmacological effects of the mammalian and the amphibian peptide.

We have shown that the i.p administration of BN and GRP-27 (same doses used in this study) reduce the MS and prolong the IMI [36]. Here, the i.v infusion of only GRP-27

reduced the MS; BN failed to evoke this response. However, the i.v infusion of BN prolonged the IMI. Although we have not measured the plasma levels of GRP, the finding that only the highest dose of GRP-27 prolonged the IMI whereas all doses reduced the MS may indicate that most likely this prolongation is a pharmacological response rather than a physiological one. Taken together, the two studies suggest that the peptides reduce the MS and prolong the IMI length at different sites because each route evoked different responses. We speculate that GRP-27 and BN administered via i.p activated the BB₁ receptor in multiple locations that regulate the MS and IMI length, whereas the GRP-27 and BN administered via i.v reached these receptors in at least two different locations, one of which regulates the MS by GRP-27, while the other controls the IMI length by BN.

Multiple lines of evidence indicate the gastrointestinal tract as a possible location for the sites of action that regulates the MS and the IMI length by GRP. First, GRP is secreted by the enteric neurons of the stomach and intestine [2, 3, 20-22, 27, 32]. Second, although the distribution of the GRP receptor is not yet available, we have shown utilizing a real-time polymerase chain reaction that the distribution of GRP is mostly in the ileum > cecum > distal duodenum > pyloric sphincter > colon > distal stomach > esophagus > jejunum > proximal duodenum > rectum > proximal stomach = medulla of the hindbrain [25]. Third, the gastrointestinal tract contains both vagal and spinal afferents, which are required, at least in the BN and GRP-29 cases, to carry the satiety and satiation signals by GRP to the hindbrain [28, 31, 37]. Finally, we [26] and others [8] have shown that the infusion of BN and GRP-29 (the large molecular form of GRP that exists only in rats) into the celiac artery, which supplies the stomach and upper duodenum, reduces the MS and prolongs the IMI length more than intraperitoneal injections or infusions in the cranial mesenteric artery, which supplies the small intestine and part of the large intestine. Therefore, the data above collectively suggest that the site(s) of action that regulates the reduction of the MS and the prolonging of the IMI resides in the gastrointestinal tract. Further work, however, is required to identify this hypothetical site(s).

Contrary to what has been shown by Rushing and colleagues [23], in the current work, the i.v administration of BN failed to reduce the MS but was able to prolong the IMI. The two experiments differ in many aspects. First, the previous work measured rat chow, whereas this work measured a hedonic meal, sucrose. The previous work measured food intake in a non-disturbed rat model; the current work measured food intake in overnight food-deprived rats. The previous work tested high doses of BN (1.3, 2.6 and 5.2 nmol/kg). The current work tested BN doses of 0.21, 0.41, and 1.03 nmol/kg. These factors, especially the high doses of BN used in the Rushing experiment, may have contributed to the differences in the results of the two studies. The high doses of BN used in the previous study [23] may have leaked through the blood-brain barrier or activated vagal and spinal afferents locally – at the gastrointestinal level – to reach the feeding control areas in the hindbrain and cause the reduction of the MS. In addition, BN is thirteen times more potent in binding the BB₁ receptors than is GRP [25]. Therefore, the activation of these receptors by BN will last longer than when they are activated by GRP, resulting in the prolonging of the IMI. Furthermore, the pharmacology of the amphibian peptide is most likely different than that of the mammalian one. Bombesin may be activating an unrevealed subset of BB receptors plausibly in the gastrointestinal tract as discussed above. These possibilities may explain the

finding that only one dose of BN (0.21 nmol/kg), the smallest tested dose in this study, was able to reduce the size of the second meal (Figure 4). Again, because BN does not exist in mammals, the pharmacology of this peptide is different, which may have caused the current results. However, we cannot find an explanation for the lack of this response to the two higher doses of 0.41 and 1.03 nmol/kg. These results require further testing.

In conclusion, the current work highlights the possibility that the MS and IMI, in response to GRP, can be regulated by two different sites, most likely in the gastrointestinal tract.

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Highlights

I.P administration of GRP-27 and bombesin reduce meal size and prolongs IMI

We hypothesized that the I.V administration of GRP-27 and BN will be as effective

We found that GRP-27 reduces only MS and BN only prolonged the IMI

I.V administration of GRP-27 and BN affected the MS and IMI different than I.P

This suggests that the MS and IMI are regulated at different sites

Figure 1. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the first meal size

Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats (n=8), and the first meal size (MS, 10% sucrose) was determined. Only GRP-27 (all doses) reduced the MS relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN

Figure 2. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the length of the intermeal interval

Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats (n=8), and the time between the first and the second meals (intermeal interval, IMI) was determined. GRP-27 (1.03 nmol/kg) and BN (all doses) prolonged the IMI relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN

Figure 3. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the satiety ratio

Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats (n=8), and the satiety ratio (SR; the time between two meals (the intermeal interval in minutes) divided by the first meal size in ml) was determined. GRP-27 (1.03 nmol/kg) and BN (0.21 and 1.03 nmol/kg) increased the SR relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$), and BN at 1.03 nmol/kg increased this ratio more than the same dose of GRP-27 († denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN

Figure 4. Effect of the intravenous infusion of gastrin-releasing peptide-27 and bombesin on the size of the second meal

Gastrin-releasing peptide-27 (GRP-27) and bombesin (BN) (0, 0.21, 0.41 and 1.03 nmol/kg) were infused in the femoral vein of overnight food-deprived Sprague Dawley rats (n=8), and the second meal size (MS, 10% sucrose) was determined. Only BN (0.21 nmol/kg) reduced the second MS relative to the effect of the saline vehicle (* denotes the significance, $p < 0.05$). Black bar: saline, empty bars: GRP-27, gray bars: BN